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SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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Published by the American Pharmaceutical Association. Publication office, 20th and Northampton Sts., Easton, Pa. Editorial office: Scientific Edition, 32 S. Greene St., Baltimore, Md. Practical Pharmacy Edition: 2215 Constitution Ave., Washington, D. C.

Annual Subscription: Journal of the American Pharmaceutical Association, complete $5.00; Canada, $6.00; Other Foreign, $6.50. (*a*) Scientific Edition, $4.00; Canada, $4.35; Other Foreign, $4.50; Single Numbers, $0.35; Canada, $0.40; Other Foreign, $0.50. (*b*) Practical Pharmacy Edition, $1.50; Canada, $1.80; Other Foreign, $2.00; Single Numbers, $0.15; Canada, $0.20; Other Foreign, $0.25.

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Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year: Scientific Edition monthly on the fifth. Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

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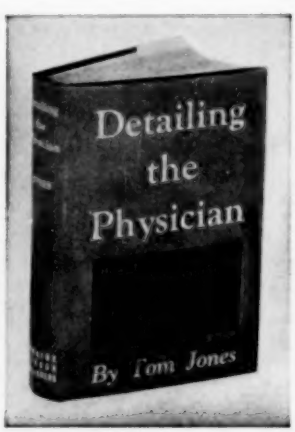

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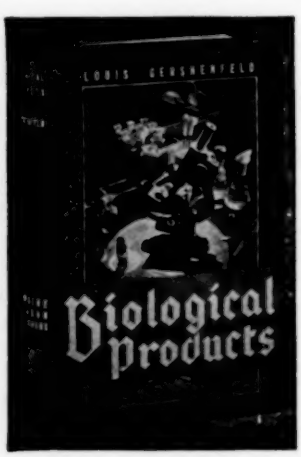

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Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

A. G. DuMez, Editor, Baltimore, Maryland

VOLUME XXX | *SEPTEMBER, 1941* | NUMBER 9 CONSECUTIVE No. 17

Physical Evidence of Association of Camphor with Phenol and the Cresols

*By Alfred W. Francis**

The loss of the corrosive action of phenol when mixed with camphor has been known and employed in treating local surgical infections for more then forty years. Phenol-camphor mixtures produce no irritation on sound tissues and may even be used in open wounds. It is difficult to explain this observation except by assuming some combination between phenol and camphor. Yet any method of chemical analysis for phenol reveals the total percentage in the mixture. Even water in sufficient amount, decomposes the compound, if any, dissolving the phenol and causing separation of solid camphor.

The present investigation was undertaken to show physical evidence for the probable presence or absence of compound formation in mixtures of camphor with phenol and the three cresols, respectively. The latter were of particular interest because they possess some advantages in bactericidal properties over the phenol-camphor mixtures, and because no investigation of those systems has been published.[1]

Günther and Peiser (1) made a somewhat similar study of the phenol–camphor system. They determined the freezing curve of the system, and also the indices of refraction, densities and solubilities in water of several mixtures of phenol and camphor. Although the freezing curve revealed a complex melting at −13.7° C., the flatness of its curve indicated substantial decomposition on melting; and the curve for density indicated no contraction on mixing. They concluded that no complex existed in the mixture at room temperature, but that the non-corrosive property of the phenol was due to the solvent action of the camphor phase, which permitted only a low concentration of phenol in body fluids.

The distribution of phenol between the camphor phase and water which they studied, is the most direct method of attack; and their conclusion based upon their data alone is logical. But in connection with additional data presented here, a different interpretation is probable. This is suggested by the fact that phenol has nearly the normal distribution between water and benzene,

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[1] This use of cresols and camphor has been covered by U. S. Pat. 1,924,169 of J. E. Stacey and assigned to Otis Clapp and Son, Inc.

although the latter is an excellent solvent for it and is related to it in structure more closely than is camphor, which shows an abnormal affinity for phenol in equilibrium with water.

The liquefaction of camphor and phenol on mixing was observed by Buffalini (2) in 1873. The freezing curve has been studied by Wood and Scott (3) and by Kremann, Wischo and Paul (4) and partly by Leger (5) as well as by Günther and Peiser (1). Kremann, Wischo and Paul failed to find a complex crystallizing out because of the jelly-like consistency of the liquid mixtures at low temperatures. Wood and Scott found a melting temperature for the complex of −18.6° C., but their curve was otherwise in fair agreement with that of Günther and Peiser.

EXPERIMENTAL

Preparation of Material.—About 500 Gm. each of phenol (Recryst.) and three cresols (Pract.) were obtained from the Eastman Kodak Company and distilled with an ordinary flask and air condenser, rejecting the first and last 50 cc. The main portion was collected over a 1.5° C. range, and this range always included the boiling point given in the International Critical Tables for the desired product, namely, 180° C. for phenol, 190.8° C. for *o*-cresol, 202.8° C. for *m*-cresol and 201.8° C. for *p*-cresol. The *m*-cresol froze completely at 11.0° to 10.8° C., somewhat higher than the 10° C. given in the tables The other compounds were allowed to freeze about half and were then decanted. The residual crystals showed freezing temperatures of 40.8°, 30.2° and 34.8° as compared with those in the tables—41°, 30.1° and 34.8°, respectively. Since the only probable impurities, water and the other cresols, would lower the freezing points (6) (except for *p*-cresol in *m*-cresol) the materials were considered sufficiently pure. Additional proof was shown by the bromine titrations (7) which showed the following number of moles of bromine per molecular weight:

	Calcd.	Found
Phenol	3.0	2.993
m-Cresol	3.0	3.007
o-Cresol	2.0	1.998
p-Cresol	2.0	2.006

A good grade of gum camphor was distilled rapidly without a thermometer through a short air condenser into a large evaporating dish covered by a towel to prevent excessive loss by sublimation. This crude technique was necessitated by the high melting point and hard gummy nature of the solidified liquid. The product was ground and sifted through a 20-mesh screen for convenience in making mixtures. It melted at 178.6°, which is near the figure given in International Critical Tables, 179°.

Freezing Curves.—The initial freezing temperatures of various mixtures of camphor with phenol and the cresols were determined by the visual method, using about 10 Gm. of material in test-tubes. The temperatures above −5° C. were read by means of a set of short stem 50° range Anschutz thermometers graduated in fifths of degrees. The

Table I.—Initial Freezing Temperatures of Mixtures of Camphor with Phenol and the Cresols

(Percentages in Mole Per Cent of Camphor, Temperature in Degrees Centigrade)

Phenol		*o*-Cresol		*m* Cresol		*p* Cresol	
Per Cent	Temp.	Per Cent	Temp.	Per Cent	Temp.	Per Cent	Temp.
0	40.8	0	30.2	0	11.0	0	34.8
8.25	33.2	4.82	27.9	5.95	6	10.07	28.7
14.02	25.3	8.89	24.7	10.7	1	16.75	19.4
18.27	18.1	11.3	22.8	14.84	− 4	21.45	13.3
21.39	11.3	12.27	22	17.96	− 7	25.00	4.5
24.0	6.5	17.53	16	22.6	−12	27.80	− 3
25.0	4	18.06	16	25	−19	28.7	− 3
27.5	− 4	21.7	11			30	− 7
30	−10	23.1	11				
32.5	−24	25.0	7	67.7	+35	61.65	− 4
35	−22	26.05	6	71.65	65	64.75	+19
42	−16	29.3	0	76.0	89	71.5	60
50	−12	30	− 3	80.9	116	74.2	80
56	−12	32.95	− 9	86.7	140	79.25	106
59	−14	33.3	−22[a]	93.45	162	85.85	136
60.75	−17.5			100	178.6	92.1	158
62.1	−11	43.5	−19				
63.58	− 1	50	−18				
65.4	+17	60	−19				
67.8	38	63.65	+12				
71.35	64	67.7	36				
75.4	86	71.6	64				
81.25	118	79.4	107				
88.81	145	85.55	138				
		92.1	159				

[a] Metastable.

lower range was observed with a toluene thermometer with a range from −50° to +55° C. It read 0.0° in an ice and water bath, as did the lowest Anschutz thermometer. The observations are recorded in Table I.

Figure 1 shows the phenol-camphor curve in comparison with those of Wood and Scott and of Günther and Peiser. It is slightly higher than either in most places, probably because of purer material. Günther and Peiser admitted the presence of 0.23% water in their phenol. The equimolecular complex melts at −12° C.

Figure 2 shows the curves for camphor with the three cresols. The *o*-cresol curve shows the existence of an equimolecular complex melting at −18°, quite analogous to that of phenol and camphor. The metastable point was found by seeding the supercooled 33.3 mole per cent mixture with the equimolecular complex. It was not found possible to crystallize out the corresponding complexes of camphor with *m*- and *p*-cresols because of the extreme viscosity of the mixtures at temperatures below −20°. Samples of 33.3 mole per cent and of 50 mole per cent camphor with each were maintained at −30° to −40° C. for several hours with occasional scratching with a broken glass rod. They were also seeded with crystals of the *o*-cresol and the phenol complexes, suspecting them to be isomorphous, but the mixtures remained thick transparent jellies. Nevertheless, other evidence presented later makes the existence of these complexes in solution probable, even though they could not be crystallized. In equilibrium with solid camphor, mixtures with the three cresols give an identical freezing curve, which is nearly the same for phenol mixtures. Compare also salicylic acid-camphor complexes (8).

Densities.—Günther and Peiser (1) determined the densities of certain liquid mixtures of phenol and camphor, presumably with the idea of detecting contractions on mixing, which would be evidence of combination. If this was their object, their plot of density against *mole per cent*, which gave them a concave curve, is without significance. The plot of density should have been against per cent by *volume* in order to detect contraction by deviation from a straight line; but since the precise density for liquid camphor at room temperature (hypothetical substance) is unknown, this cannot be done. The alternative of plotting specific volume against per cent by weight is just as satisfactory for the purpose. To detect small contractions the plot should extend over the maximum range of liquid mixtures available (they covered little more than half the range), and to make the range wider a higher temperature, 25° C., is preferable to their 15.8° C.

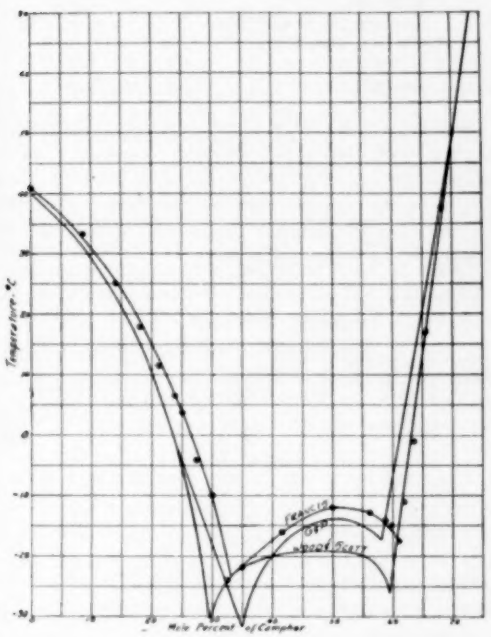


Fig. 1.—Freezing Curves—Camphor-Phenol.

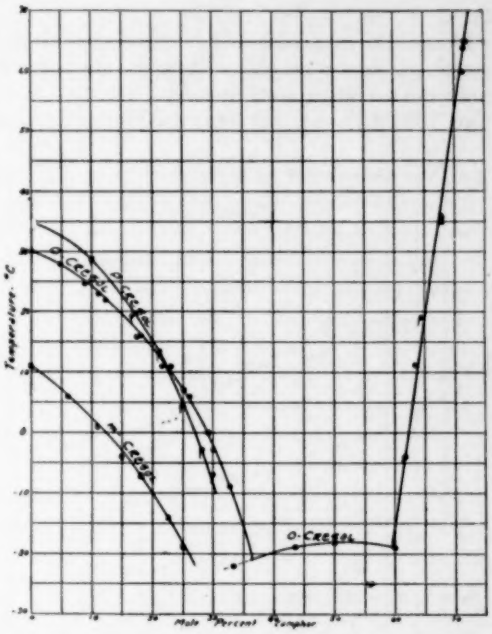


Fig. 2.—Freezing Curves—Camphor Cresols.

Densities of mixtures of camphor with phenol and with all three cresols are recorded in Table II. They were determined in a 2-ml. pycnometer at room temperature, 24–27° C., and corrected to 25° C. by means of the temperature coefficients (about 0.08% per degree (9)). The pycnometer was calibrated with water giving 2.0106 and 2.0127 ml. and with mercury giving 2.0099 and 2.0137 ml., mean 2.0117 ml.

The values for phenol and *p*-cresol are extrapolated. Those for all four phenols agree fairly well with those determined or estimated in the literature (9), 1.0713, 1.0440, 1.0302 and 1.0307, respectively.

Table II.—Densities of Mixtures of Camphor with Phenol and the Cresols

(Percentages in Wt. Per Cent of Camphor, Densities at 25° C. in Gm. per Ml.)

Phenol		o-Cresol		m-Cresol		p-Cresol	
(0	1.0724)	0	1.0423	0	1.0270	(0	1.0299)
22.4	1.0465	10.3	1.0311	10.2	1.0200	10.1	1.0225
22.8	1.0459	23.25	1.0213	19.6	1.0131	23.5	1.0123
32.7	1.0350	32.6	1.0130	29.2	1.0065	33.2	1.0056
40.2	1.0258	43.1	1.0047	38.45	0.9995	43.9	0.9982
41.0	1.0235	53.0	0.9952	48.4	0.9930	54.55	0.9905
44.7	1.0193	58.5	0.9902	58.4	0.9860	63.8	0.9838
56.5	1.0075	64.0	0.9855	66.4	0.9800	72.0	0.9768
61.8	1.0005	74.8	0.9766	74.6	0.9745		
70.25	0.9922						
75.4	0.9860						
76.5	0.9840						

But the densities observed by Günther and Peiser (1) are entirely inconsistent with Table II and with the value for phenol in International Critical Tables (9), being lower at 15.8° C. than those observed at 25° C.

Plots of the reciprocals or specific volumes against weight per cent were almost straight. In order to make the curvature more apparent, a derived function, $1000/d_4^{25}$—wt. per cent, is plotted against

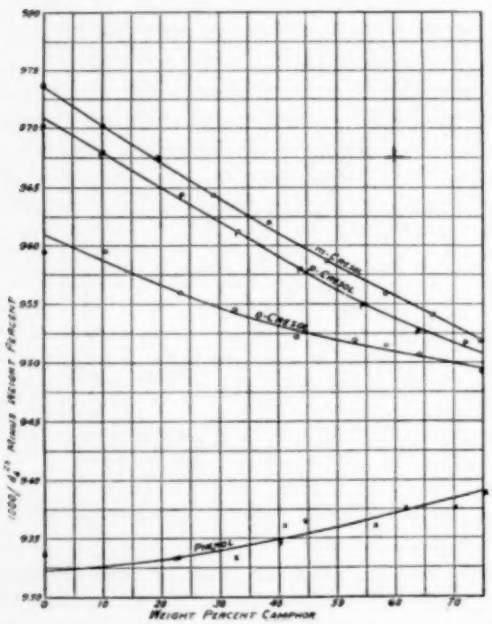


Fig. 3.—Contractions of Liquid Mixtures of Camphor with Phenol and the Cresols.

weight per cent in Fig. 3. The contraction indicated by the concavity of the curves is about 0.25% for the range covered. It would be about 0.5% for the full range, 0–100%, if available.

In view of the equivocal results of Günther and Peiser (1) on indices of refraction, this property did not seem to promise much information, and so was not used in the present investigation.

Partial Solubility in Water.—Pure phenol has a solubility in water of 8.45% at 25° C. In contact with 50 mole per cent solutions of phenol in various solvents water contains the following percentages of phenol (10):

Solvent	Wt. Per Cent in Water
Benzene	4.53
Carbon tetrachloride	4.1
Carbon disulfide	5.2
Chloroform	4.25
Bromoform	4.84

These figures all show about half the solubility of pure phenol, indicating that the solubility is probably roughly proportional to the mole fraction unless there is some chemical combination with the solvent. In the case of camphor the partial solubilities are shown in the following table.

Table III.—Distribution of Phenol between the Camphor Phase and Water

Mole Fraction of Phenol in Camphor Phase	Wt. Per Cent of Phenol in Water Phase	
	Günther and Peiser (1)	Francis
0.35	..	0.65
0.373	0.61	..
0.421	0.79	..
0.500	1.30	1.40
0.616	2.25	..
0.649	2.82	..
0.699	3.45	..

The equimolecular mixture (38.2% by weight) shows a solubility of only 1.3% or 1.4%, less than one-third as much as would be expected from the values for other solvents. This seems to indicate that at least two-thirds of the phenol is combined

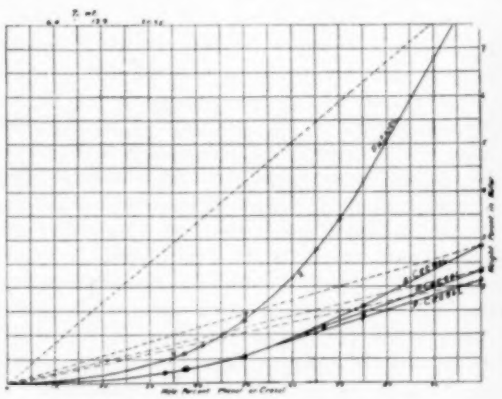


Fig. 4.—Distribution of Phenol or Cresol between Camphor Phase and Water.

in some way with the camphor. Similar tests were made with mixtures of camphor with the cresols. In each case 10 cc. of the mixture and 10 cc. of water were shaken thoroughly in a thick-walled text-tube and then centrifuged. A sample of the clear aqueous layer (in some cases the upper and in some cases the lower) was withdrawn with a pipette, weighed and titrated with bromide-bromate solution (7). Phenol and *m*-cresol consume three moles of bromine per mole and *o*- and *p*-cresols two each. Under the conditions of titration camphor consumes no bromine. The results are given in Table IV and Fig. 4.

Table IV.—Distribution of Cresols between the Camphor Phase and Water

Mole Fraction Cresol	Wt. Per Cent in Water		
	Ortho	Meta	Para
1.00	2.88	2.36	2.14
0.75	1.59	..	1.42
0.667	..	1.18	..
0.50	0.59	0.54	0.57
0.375	0.29	..	0.34
0.333	..	0.24	..
0.50 in benzene	1.69	1.32	1.20

As in the case of phenol, the equimolecular mixture in benzene showed in each case a solubility a little more than half that for the pure cresol; but the equimolecular mixture in camphor shows only about one-quarter as much. Apparently more than half the cresol is combined with camphor in each case.

Partial Vapor Pressures.—Another method of measuring the thermodynamic activity of one component in a mixture is by means of partial vapor pressures. This would be more nearly rigorous than that of solubility, studied in the last section; but it is usually more difficult to measure accurately. In the systems under investigation the vapor pressures of all the components at room temperature are so low that an accurate measurement of partial pressures at low temperatures is practically impossible. On the other hand, the fact that the vapor pressures of all the components are comparable and the fact that we have a simple and accurate method of analysis of mixtures (that of bromination) make such a study promising. The study was made at approximately 100° C., at which the several vapor pressures are as follows (11):

Camphor solid	20.0 mm.	*o*-Cresol	31.6 mm.
Camphor liquid	30.7	*m*-Cresol	19.05
Phenol	43.4	*p*-Cresol	18.3

The value for liquid camphor was computed from the equation $\log p = 7.692 - (2316/T)$ derived from the vapor pressures at higher temperatures. This value was used in the partial pressure experiments.

Two "railroad" gas bubbling tubes of Pyrex glass were modified slightly as shown in Fig. 5. Two small glass tubes were bent as in (*a*) and (*b*), respectively, so that when the two parts were connected at (*c*), an air stream from a calcium chloride drying tube bubbled through the first railroad tube, then through the first narrow tube, back into the second railroad tube and out through the second narrow tube to the water pump. The railroad tubes were fastened together with a rubber band and immersed in a beaker of boiling water, while the narrow tubes served as condensers, and were immersed in ice water. Bulbs at the bottoms of the bends received the distillates. It was found necessary to have the exits from the railroad tubes below the boiling water and to have initial dips in the receiver tubes to prevent refluxing back into the railroad tubes. A small portion of the distillate was collected in these dips. Each of the four tubes was provided with a wire with a loop to permit weighing in an analytical balance.

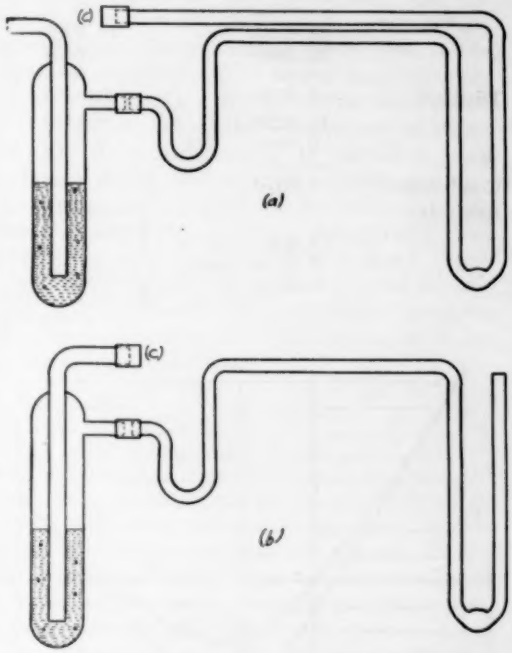


Fig. 5.—Partial Vapor-Pressure Apparatus.

In each run 10–12 cc. of the desired mixture of camphor with phenol or a cresol were put into railroad tube (*b*) and an equal amount of the same cresol in (*a*). Since exactly the same air was passed through each of the two liquids which were at exactly the same temperature (assumed to be 100° C., but which might have been slightly lower occasionally), the relative amounts of cresol or phenol vaporized and condensed from the two tubes are a fairly accurate measure of the relative partial pressures. The volume of air was not measured, so that only relative values were obtained. A run lasted about an hour and vaporized about half a gram of each liquid. This must have required about eight liters of air. Each tube was weighed before and afterward to compare the gain for the condensers with the loss from the railroad tubes. These usually checked, respectively, within 5%. The condenser tubes were then rinsed out carefully into 300-cc. Erlenmeyer flasks mostly by means of water but

using a little methanol. After being diluted to about 150 cc. the contents of the flasks were titrated with bromide-bromate (7) to determine cresol (or phenol). In the case of the (*b*) tubes the remainder of the condensate was considered camphor. The condensate in (*a*) was titrated for completeness, but was always close to 100% cresol. When a phenol-camphor mixture was used, the reference liquid was not phenol, which would solidify and plug the receiving tube, but *o*-cresol. All the cresols supercooled enough so as to give no trouble in this respect.

The result of a typical run was as follows:

Mixture in (*b*) 50 mole per cent of camphor and phenol. Reference liquid in (*a*) *o*-cresol.

	(*a*)	(*b*)
	43.6335	41.8908
	42.5605	41.2454
Distilled	1.0730	0.6454
	10.9220	8.8430
	9.8850	8.2423
Condensate	1.0370	0.6007
Analysis	1.038 Gm. = 9.6 m. moles *o*-cresol	0.248 Gm. = 2.64 m. moles phenol 0.353 Gm. = 2.32 m. moles camphor
Vapor pressure	31.6 mm.	8.7 mm. phenol 7.65 mm. camphor

If Raoult's law were obeyed for this mixture the partial pressures would have been 21.7 mm. phenol and 15.35 mm. camphor (half the values for pure components). The lower observed values indicate a holding back of both components (phenol somewhat more than camphor) probably because of a less volatile complex of the two components in the mixture. To be sure, Raoult's law fails in many other cases, but I believe that where deviations are substantial, as in this case, it is partial evidence for more or less stable association of the components. In this case the greater retention of phenol than of camphor might mean a greater proportion of phenol combined, that is some C-P_2 as well as C-P. But the mixture containing 66.7 mole per cent phenol shows a considerable excess of the latter, indicating that the total composition of all the combined portion is between one-half and two-thirds phenol.

o-Cresol gives about the same result as does phenol. *m*- And *p*-cresol, however, seem to indicate nothing but compounds of camphor with two moles of cresol, since such mixtures give distillates of nearly the same composition; while the 50 mole

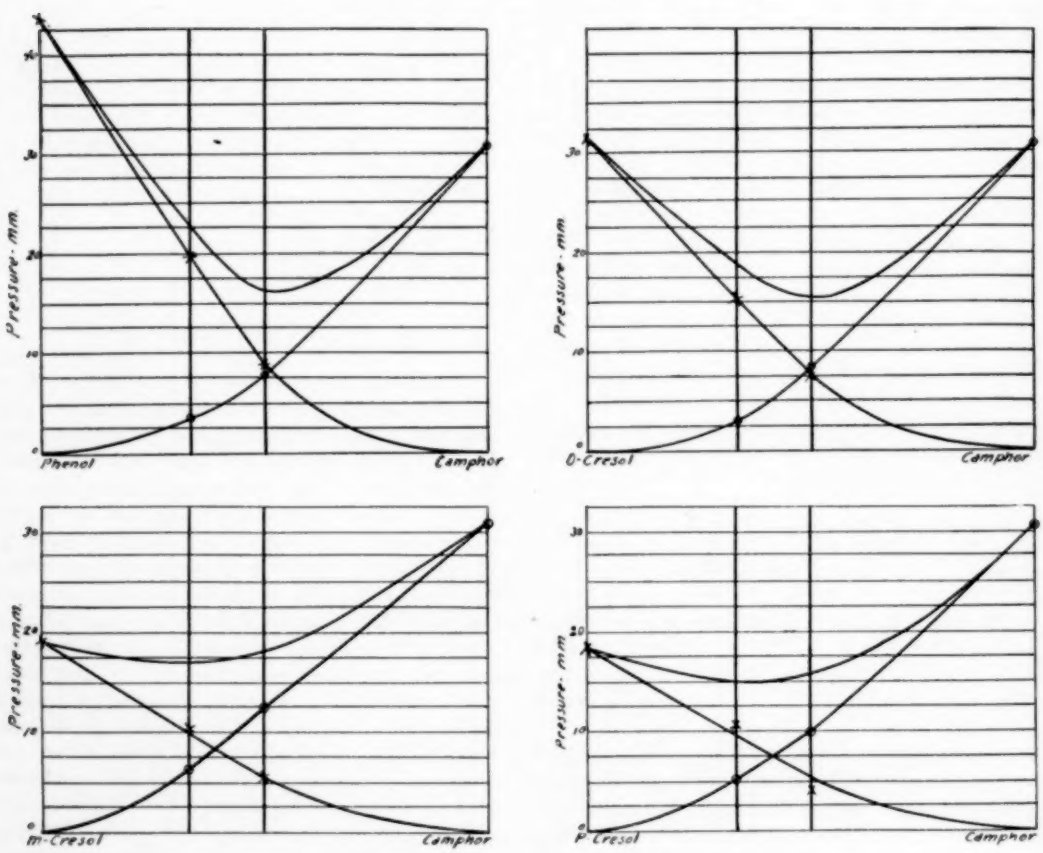


Fig. 6.—Partial Vapor Pressure at 100° C.

per cent mixtures evolve such an excess of camphor that the distillate contains a slush of camphor crystals. Additional conclusions will be discussed in relation to other observations later. A summary of the complete results is given in Table V, and also in Fig. 6.

Table V.—Partial Vapor Pressures of Camphor-Phenol and Camphor-Cresol Mixtures

Substance (Figures Are Moles)	Condensate Weight, Gm.	Condensate Millimoles	Vapor Pressure, Mm. Observed[a]	Vapor Pressure, Mm. Calcd. from Raoult's Law
o-Cresol	1.037	9.60	31.6	
1 Camphor	0.353	2.32	7.65	15.35
1 Phenol	0.248	2.64	8.7	21.7
o-Cresol	0.832	7.70	31.6	
1 Camphor	0.116	0.76	3.15	10.2
2 Phenol	0.445	4.73	19.4	28.9
o-Cresol	1.129	10.44	31.6	
1 Camphor	0.421	2.67	8.1	15.35
1 *o*-Cresol	0.263	2.44	7.35	15.8
o-Cresol	0.779	7.20	31.6	
1 Camphor	0.114	0.75	3.25	10.2
2 *o*-Cresol	0.386	3.57	15.7	21.1
m-Cresol	0.448	4.15	19.05	
1 Camphor	0.443	2.85	13.0	15.35
1 *m*-Cresol	0.131	1.21	5.5	9.5
m-Cresol	0.392	3.63	19.05	
1 Camphor	0.1875	1.23	6.4	10.2
2 *m*-Cresol	0.219	2.03	10.6	12.7
p-Cresol	0.579	5.35	18.3	
1 Camphor	0.455	2.99	10.2	15.35
1 *p*-Cresol	0.132	1.22	4.2	9.1
p-Cresol	0.498	4.61	18.3	
1 Camphor	0.201	1.32	5.2	10.2
2 *p*-Cresol	0.276	2.55	10.1	12.2

[a] For pure substances taken from International Critical Tables (11). For mixtures taken proportional to the millimoles in the condensate.

Temperature Effects on Mixing.—If solid camphor and solid phenol are mixed adiabatically, the temperature falls about 12° C. This absorption of heat is due to the heats of fusion of the two crystalline compounds. However, an estimate of these two heats of fusion indicates that the fall in temperature should be nearly twice as great, if no reaction occurred. This was confirmed by mixing two strong solutions of camphor and phenol, respectively, in benzene. In this case there was a *rise* of over 8° since there was no heat of fusion to be absorbed. This indicates qualitatively that there is considerable chemical combination between the camphor and phenol, even in benzene solution. This method seemed a promising one for estimating the relative amounts of free and combined phenol in mixtures, and also for deciding the composition of the complex.

In the more quantitative experiments the calorimeter consisted of a small Dewar flask of 50-ml. capacity. About 15 ml. of one reagent were placed in the flask and a similar amount of the other one in a tube made from an ordinary test-tube by blowing a 9-mm. hole in the bottom and drawing it down to 9 mm. at the top, the total length being 10.5 cm. The bottom hole was closed by a tiny loose-fitting, flat cork and the whole tube was immersed in the other reagent in the Dewar flask. When the two liquids had reached the same temperature, the cork was pushed out with the thermometer, and the tube was withdrawn over the thermometer. The mixture was stirred and the temperature read about every five seconds until the maximum was reached, usually in ten to fifteen seconds. The same Anschutz thermometer was used as was mentioned previously.

The solid components camphor and the cresols were entirely unsuited as reagents for these heat effects on mixing. A four-molar solution of camphor in benzene was chosen as one component since it was almost saturated (65.6% camphor) and was found by experiment to produce no change in temperature when further diluted with benzene in the apparatus described. The cresols are still more soluble in benzene, and because of their lower molecular weight an eight-molar solution can be made. But such a solution even in the case of *m*-cresol evolves heat on dilution with benzene and this would complicate studies on the heat of mixing with the camphor solution. Accordingly, four normal solutions of the cresols were used. These evolve only a trivial heat on dilution; and by having the two components of equal molality the choice of volumes for mixing and the calculations of the results were greatly simplified.

In the case of each cresol and phenol, mixtures of the two components were made in the proportion of one to one by volume and also one of camphor solution to two of cresol solution, these being the most probable proportions for combination as judged by the vapor-pressure studies. Then samples of these mixtures were used as reagents, mixing them with excess camphor solution or cresol solution or other mixtures, etc., observing the rise in temperature in each case. These were plotted in the manner shown in Fig. 7, with the molar composition of the two solutions as abscissa and the temperature rises as ordinates. When intermediate compositions were used, a dotted line was drawn connecting the tops of the ordinates obtained in making those mixtures; and the new ordinate was drawn from the appropriate point on the dotted line. This method was considered more accurate than that of making all the mixtures using the original components, because the temperature rises were much smaller and the exact curvature of the sloping sides of the curves was of importance in drawing the tangents, as indicated later. By drawing a line through the tops of all the ordinates a curve results which is presumably a function of the amount of complex present in any mixture. Tangents to the curve at the two ends show the amount of compound which would be present if there were no dissociation. A comparison in ordinate between the curve and the tangent shows the relative amount of phenol or cresol or camphor which is combined.

Strictly, the specific heats of the various mixtures should have been employed with the temperature

rises to obtain heat evolved, and this was tried. But the specific heats of all the solutions were nearly the same, and differences were further decreased by the heat capacity of the calorimeter, so that the effect upon the diagrams was almost negligible. Moreover, many assumptions were necessary in estimating these specific heats. It seemed probable that the use of temperature rises would give results as accurate as were warranted by the precision of the observations and by the assumption that the heat evolved was proportional to the amount of compound formed (not quite true if more than one complex exists in one system).

The results are recorded in Table VI and Fig. 7.

All four of the curves in Fig. 7 are unsymmetrical, bulging on the side of the phenol or cresol. It was suggested that this was due to association of the phenol so that the equilibrium in solution was approximately $P_2 + 2C \rightleftarrows 2PC$, but calculation showed that this would make the curve bulge on the camphor side. The ideal cryoscopic behavior of camphor permits little probability that the bulge is

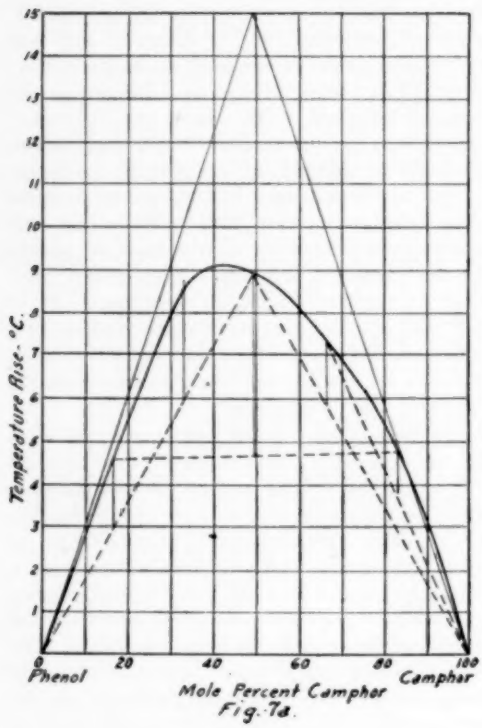


Fig. 7a.

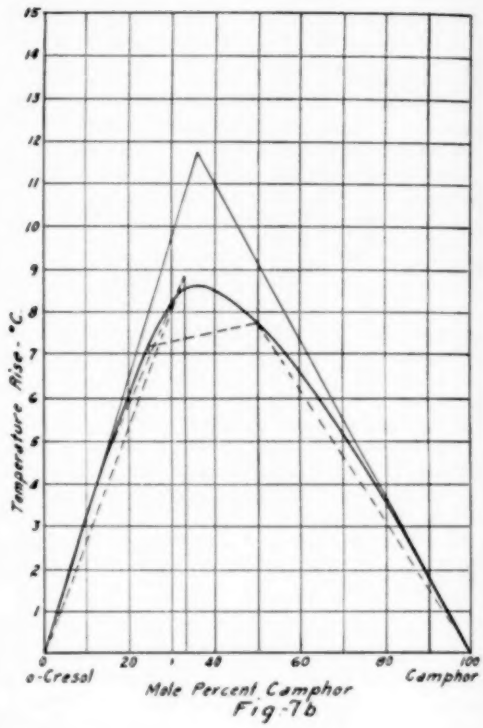


Fig. 7b

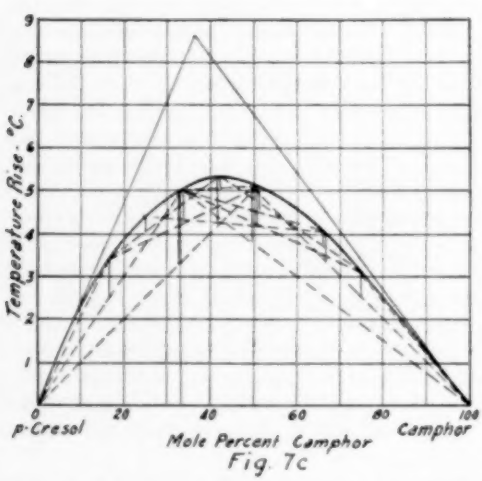


Fig. 7c

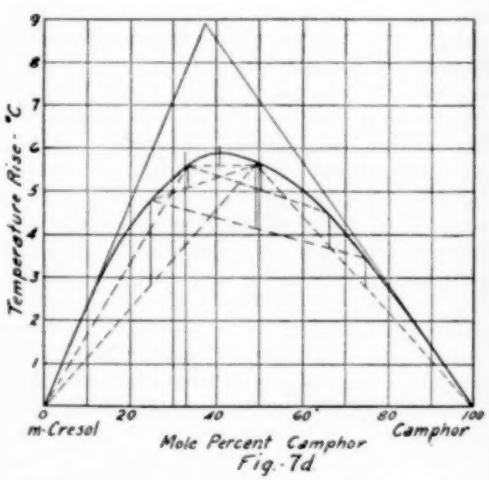


Fig. 7d

Figs. 7a–7d.—Temperature Rises on Production of Liquid Mixtures of Camphor with Phenol and the Cresols in 4*N* Benzene Solution.

Table VI.—Rises in Temperature on Producing Mixtures of Camphor with Phenol and the Cresols

Rise in degrees Centigrade
C = 4*M* solution of camphor in benzene
Ph = 4*M* solution of phenol in benzene
O = 4*M* solution of *o*-cresol in benzene
M = 4*M* solution of *m*-cresol in benzene
P = 4*M* solution of *p*-cresol in benzene
Compositions in mole per cent C

Components	Camphor-Phenol Mixture, Mole Per Cent C	Rise, °C.
Ph + C	50	8.95
C + 50[a]	66.7	1.52
Ph + 50	33.3	2.85
C + 66.7	83.3	1.10
Ph + 33.3	16.7	1.60
16.7 + 83.3[b]	50	4.50

Components	Camphor-*o*-Cresol Mixture, Mole Per Cent C	Rise, ° C.
O + C	50	7.8
O + C	33.3	8.9
C + 50	75	0.65
O + 33.3	16.7	0.75
16.7 + 33.3	25	0.75
50 + 25	33.3	1.20

Components	Camphor-*m*-Cresol Mixture, Mole Per Cent C	Rise, ° C.
M + C	50	5.6
M + C	33	5.9
M + 50	25	2.0
C + 50	75	0.7
25 + 75	50	1.5
25 + 50	33	0.5
C + 50	67	0.9
33.3 + 66.7	50	0.8
33.3 + 50	41	0.4
M + 33.3	20	1.0

Components	Camphor-*p*-Cresol Mixture, Mole Per Cent C	Rise, ° C.
P + C	50	5.08
P + C	33.3	5.02
C + 33	50	1.20
P + 33	16.7	0.85
17 + 50	33.3	0.75
C + 50	75	0.68
33 + 75	50	0.77
50 + 33	42	0.32
C + 50	67	0.85
33 + 67	50	0.58
P + 50	67	1.42
P + 17	8	0.22
C + 75	87	0.03
42 + 75	67	0.34
17 + 42	25	0.34
25 + 67	42	0.98

[a] Under components C + 50 means that 4*M* camphor solution in benzene was mixed with a benzene solution containing 2*M* camphor + 2*M* phenol, or a total of 4 moles of which 50% were camphor. These were mixed to give a final mixture containing 66.7% camphor.

[b] 16.7 + 83.3 under components means that two solutions were mixed, both of which had 4 moles solute per liter benzene. In one 16.7% of the solute was camphor and in the second 83.3%, the rest being phenol. The final mixture had 50% camphor.

due to association of camphor. The remaining possibility is that in mixtures with phenols, beside the 50 mole per cent complex, there is also a 33 mole per cent (of camphor) complex. In the case of phenol the amount of this is slight since the tangents meet at almost 50 mole per cent, but the 2:1 complex seems to predominate in the case of all three cresols. This supposition may account for the failure to crystallize out a complex at low temperature, in the case of *m*- and *p*-cresol, since its freezing curve may be below the cresol curve. This possibility may be true for *o*-cresol also, the 1:1 complex being found merely because its composition is situated more favorably between the freezing curves of the pure components. In this connection might be mentioned the "near observation" of such a complex with phenol and camphor by Leger (5) although Wood and Scott (3) concluded this was not the case. At the low temperatures required, the mixtures are so viscous that the chance crystallization of such a complex might fail of confirmation, especially if its curve falls very close to the phenol curve, as it probably does. The present investigation also failed to find such a complex by its effect on the freezing curves.

It will be noted that the vapor-pressure method seemed to indicate that as in the case of phenol only one molecule of *o*-cresol is combined with camphor, while the temperature effect indicates a 2:1 complex. The difference is due probably to the difference in temperature used. The 2:1 complex may be largely dissociated at 100° C.

The curves of Fig. 7 show also that the phenol-camphor complexes have much the highest heat of formation as judged by the height of the tangent intersection, but also the highest percentage of dissociation, about 40% at the maximum point. The *o*-cresol complexes are only slightly dissociated, about 27% at the peak. Those of *m*- and *p*-cresol are very similar to each other, with the lowest heat of formation, and about 35% maximum dissociation. It was supposed that all these percentages would be affected somewhat by the solvent benzene, which was present when the curves were estimated, but this effect must be almost negligible, since a phenol-camphor mixture (without benzene) showed no detectable heat effect when diluted with benzene. Attempts were made to compute dissociation constants for some tentative equilibrium such as $2P + C \rightleftarrows P_2C$, but these failed to give calculated curves which agreed perfectly with the observed curves, probably because more than one complex exists in solution, and because phenol and the cresols are themselves associated to an indefinite degree.

Table VII.—Freezing Temperatures of Benzene Solutions of Camphor, Phenol, the Cresols and Some Mixtures

(Concentrations in Moles per 1000 Gm. Benzene)

Camphor		Phenol		*o*-Cresol		*m*-Cresol		*p*-Cresol	
Conc.	Temp.	Conc.	Temp.	Conc.	Temp.	Conc.	Temp.	Conc.	Temp.
0	5.25	0.155	4.85	0.54	2.85	0.43	3.25	0.525	3.15
0.31	3.65	0.31	4.40	1.08	0.88	0.65	2.65	1.05	1.80
1.00	0.13	0.62	3.65	1.62	−0.25	0.85	2.05	1.58	0.80
1.52	−2.20	1.22	2.70	2.16	−1.50	1.08	1.50	2.10	0.00
		2.56	0.60	3.24	−3.60	1.29	1.05	2.63	−1.00
		3.86	−1.20	4.32	−5.60	1.51	0.60	3.15	−1.85
		5.18	−3.0	5.40	−7.60	1.72	0.20	3.68	−2.50
				6.48	−9.5	2.16	−0.20	5.1	−4.20
						3.24	−1.45	6.5	−6.0
						4.32	−2.6	7.9	−6.6
						5.40	−4.2	9.3	−8.0
						6.48	−4.9		
						7.56	−6.2		

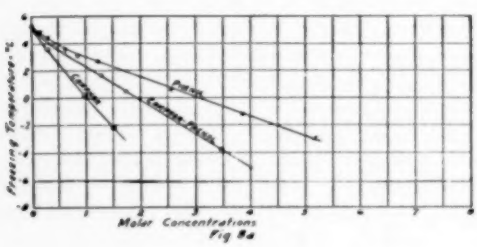
Equimolecular mixtures.

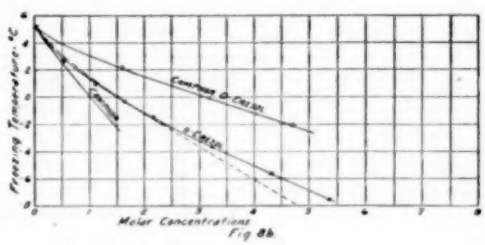
Concentrations as if not combined, *i. e.*, mean mol. wt. {123 for camphor-phenol; 130 for camphor-cresols

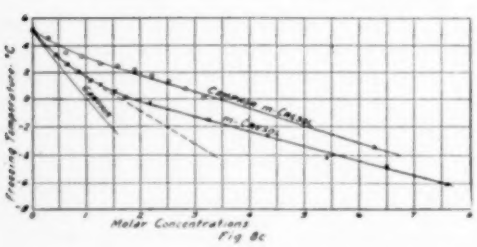
Camphor-Phenol		Camphor-*o*-Cresol		Camphor-*m*-Cresol		Camphor-*p*-Cresol	
Conc.	Temp.	Conc.	Temp.	Conc.	Temp.	Conc.	Temp.
0.43	3.70	1.57	2.15	0.315	4.65	1.57	2.35
0.62	3.35	4.72	−2.00	0.63	3.75	3.15	0.35
0.87	2.60	6.30	−4.05	0.94	3.25	4.72	−1.60
1.30	1.60	7.86	−6.00	1.26	2.70	6.30	−3.80
1.74	0.50	9.45	−7.60	1.57	2.50	7.86	−5.60
3.48	−3.80			1.89	2.20		
				2.20	1.70		
				2.52	1.30		
				2.83	0.80		
				3.15	0.25		
				6.3	−3.55		

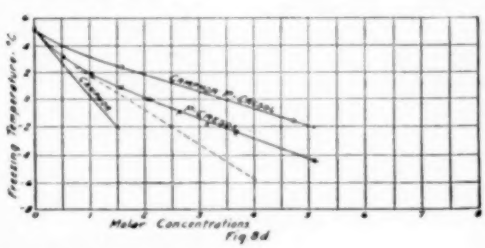
Although the maximum dissociation of the complexes seems to be 27% to 40%, judging from these curves, it will be noted that the equimolecular mixtures in the case of the cresols contain a much smaller proportion of free cresol, about 15, 23 and 21% of the total cresol, respectively. This means about 6% to 9% by weight in the mixture instead of 41.5% as found by chemical analysis. The equimolecular mixture of phenol and camphor contains 38.2% total phenol and about 15% free phenol. This indicates one advantage of cresol mixtures over the phenol mixture, since they are probably less irritating. If an excess of camphor is present, as is quite common, the percentage of free cresol is still less. Thus a mixture containing 20% total *m*-cresol by weight (26 mole per cent) seems to contain about 1½% free *m*-cresol.

Cryoscopic Molecular Weight Determinations.—The freezing temperatures were determined for solutions in benzene of camphor, phenol and the cresols,









Figs. 8*a*–8*d*.—Freezing Temperatures of Benzene Solutions.

and also of equimolecular mixtures of these, in the hope of estimating the mean molecular weight of mixtures and thus giving light from still another angle upon the question of complex formation. Although camphor shows a perfectly normal effect upon the freezing point of benzene, phenol and the cresols show abnormally high molecular weights, due undoubtedly to association, even in dilute solution. Nevertheless, the depression caused by mixtures with camphor is always considerably less than the combined depression caused by its components, although greater than either alone—indicating pronounced, though not complete association of camphor with phenol and the cresols.

These results are presented in Table VII and Fig. 8.

The camphor-phenol curve is higher than the mean between those of camphor and phenol and so indicates a greater degree of combination than that of association of the phenol alone. The camphor-cresol curves are all above those of the corresponding cresols, indicating a still greater degree of combination. If the molar concentration were computed as for the combined molecule (*i. e.*, 260 mol. wt. instead of 130), the curves (dotted lines in the figure) would fall below the cresol curves, showing incomplete combination, although in the case of *o*-cresol the curves become practically coincident at low concentrations, indicating nearly complete association or else an appreciable amount of molecules of still higher molecular weight such as a complex of one camphor with two *o*-cresol molecules.

If we compare the concentrations required to depress the freezing temperature of benzene the theoretical molar amount, 5.12°, that is, to +0.13° C., the apparent molecular weights are as follows:

Table VIII.—Apparent Molecular Weights in Benzene Solutions Freezing at +0.13° C.

Solute	Molecular Weight Observed	Molecular Weight Calcd.	Simple Moles per Complex
Camphor	152	152	1.00
Phenol	273	94	2.90
o-Cresol	156	108	1.44
m-Cresol	195	108	1.80
p-Cresol	218	108	2.015
Camphor-phenol	232	123	1.89
Camphor-*o*-cresol	397	130	3.05
Camphor-*m*-cresol	418	130	3.22
Camphor-*p*-cresol	433	130	3.33

The fact that the last three values are above 3.0 does not mean a higher complex than one of camphor with two of cresol, but only that most of the free cresol is also in aggregates.

Relationship between Free and Combined Metacresol.—The data given in the preceding sections show that there is certainly compound formation between camphor and phenol or cresol. In any mixture of cresol and camphor some of the cresol is free and some is combined. It is very desirable to know the relationship between them. The data in the preceding sections give us a means of calculating this relationship. The precision of the calculation is not high, but is sufficient to give a good idea of the extent to which combination takes place. Table IX and the curve as shown in Fig. 9 exemplify the relation in the case of *m*-cresol and are based in the first place on equilibrium constants and then checked against the data obtained in other ways, particularly the curves of Fig. 7.

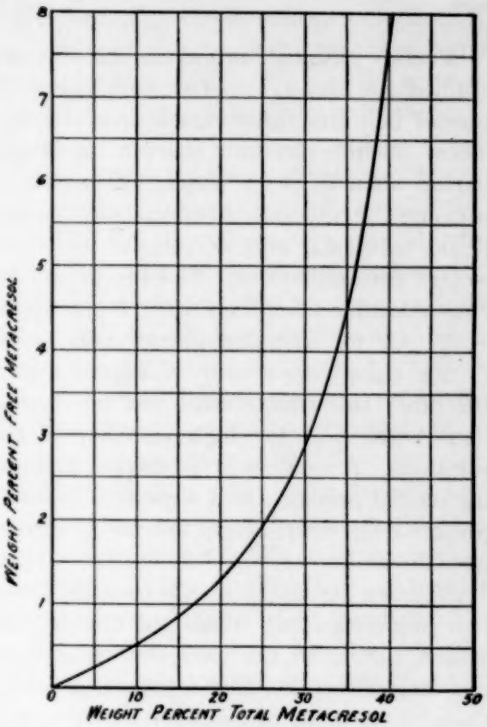


Fig. 9.—Relationship Between Free and Total Metacresol in Mixtures with Camphor.

Table IX.—Relationship between Free and Total Metacresol in Mixtures with Camphor Expressed in Weight Per Cent

Total *m*-Cresol	Free *m*-Cresol	Total *m*-Cresol	Free *m*-Cresol
3.6	0.17	24.1	1.81
7.0	0.34	26.7	2.22
10.2	0.53	29.4	2.71
13.2	0.73	31.9	3.41
16.1	0.95	35.0	4.52
18.9	1.19	41.5	8.70
21.5	1.48		

The equimolecular constant was computed from the dissociation in the equilibrium mixture and taken as 0.046 and the mass law equation as

$$\frac{xy}{1-(x+y)} = 0.046$$

where x = mol. fraction uncombined camphor
y = mol. fraction uncombined *m*-cresol

SUMMARY

Several physical properties have been studied for the systems of camphor with phenol and the three cresols, respectively. These include freezing curves, densities, partial solubilities in water, partial vapor pressures, temperature effects on mixing liquid solutions, and cryoscopic molecular weight determinations. Melting points for equimolecular complexes were found to be −12° C. for camphor-phenol and −18° C. for camphor-*o*-cresol. Complexes with the other two cresols could not be crystallized because of the high viscosity of the solutions. A convenient method of measuring partial pressures and a method of interpretation of temperature effects on mixing were devised.

Evidence derived primarily from the latter two properties, but supported also by the others, points to the existence of at least two complexes in each mixture, namely, those of one mole of camphor with one and two moles, respectively, of phenol or cresol. The former predominates in the case of phenol, but the latter in the case of all three cresols. All these complexes are partly dissociated in solution and rapidly decomposed on chemical analysis. The concentration of free cresol in an equimolecular mixture with camphor is 6% to 9%, and in a 20 weight per cent mixture with camphor the free *m*-cresol is about 1½%.

Although this investigation has been confined to the physicochemical properties of phenol-camphor and cresol-camphor mixtures, it is interesting to speculate as to the probable bearing of these properties on the known pharmacological properties of such mixtures. The low content of free phenol or cresol found probably accounts for the high toleration which wounds and tissue show to these mixtures. The fact that these are equilibrium mixtures means that they will liberate free phenol or cresol as fast as that originally present is consumed and this may explain their known antiseptic and surface anesthetic effects.

REFERENCES

(1) Günther, P., and Peiser, M., *Z. physik. Chem.*, 128 (1927), 189–202.

(2) See Lajoux, H., *Chem. Abstr.*, 11 (1917), 3376⁸.

(3) Wood, J. K., and Scott, J. D., *J. Chem. Soc.*, 97 (1910), 1573.

(4) Kremann, R., Wischo, F., and Paul, R., *Monatsh.*, 36 (1915), 911.

(5) Leger, E., *Compt. rend.*, 111 (1890), 109–111.

(6) Fox, J. J., and Barker, M. F., *J. Soc. Chem. Ind.*, 37 (1918), 270T.

(7) Francis, A. W., and Hill, A. J., *J. Am. Chem. Soc.*, 46 (1924), 2498.

(8) Le Fevre, R. J. W., and Tidemann, C. G., *J. Chem. Soc.*, (1931), 1731.

(9) International Critical Tables, Vol. III, pages 29, 33.

(10) *Ibid.*, page 428.

(11) *Ibid.*, pages 208, 221, 223, 226.

A Method for the Quantitative Determination of Theobromine or Theobromine Salts and Phenobarbital in Mixtures

*By C. W. Bell**

Due to the introduction of many new organic drugs into medicine and the continued practice of combining two or more such drugs in a single dosage form, the analysis of medicinal preparations is a problem of increasing complexity. Occasionally as many as four active ingredients may be combined in a single dosage form and it is a very common practice to combine two organic compounds in a single dosage form. In such mixtures it is necessary to have a method for the separation and quantitative determination of each of the active constituents. Much progress has been made in this branch of pharmaceutical chemistry in the past few years but a critical survey of the literature revealed that a method for the quantitative determination of theobromine, or its salts, and phenobarbital in mixtures has not as yet been reported. In attempting to develop such a method the author was primarily concerned with the analysis of tablets containing phenobarbital and Theo-

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calcin. (Theocalcin is the proprietary name for the basic calcium salicylate of theobromine, a chemical compound consisting of one mol. each of calcium, salicylic acid and theobromine. Theoretically it contains 48.15% of theobromine.) In addition to Theocalcin-phenobarbital mixtures, the method has also been successfully applied to mixtures of theobromine and phenobarbital as will be brought out later.

LITERATURE SURVEY

Although the literature does not describe a method for the determination of theobromine and phenobarbital in mixtures, there are various methods reported for the determination of the two compounds individually. These various methods will be described briefly below under the headings of "Theobromine" and "Phenobarbital" but most of the methods reported for the determination of theobromine may be also applied to the various salts and compounds of theobromine, such as Diuretin and Theocalcin.

Phenobarbital.—In most simple preparations containing only phenobarbital, the phenobarbital is extracted by the use of ether or some other suitable immiscible solvent, the solvent evaporated and the amount of extracted phenobarbital determined by weight. An example of this method is the determination of phenobarbital in the Tablets of Phenobarbital N. F. (1). In addition to the above gravimetric method there are other types of gravimetric methods depending upon the formation of complex addition products such as the complex product formed by the reaction of phenobarbital with mercuric iodide as reported by Montignie (2); the complex salts formed with iodine in an alkaline solution as reported by Bougault and Guillou (3); complex mercury salts as reported by Fleury (4), and the copper pyridinyl phenobarbital formed by the use of Zwikker's reagent (5). Most of the methods depending upon the formation of complex additional salts are unreliable due probably to the inconsistent or unknown composition of the addition product.

The accuracy of the cobalt colorimetric method proposed by Dille and Koppanyi (6) is limited to about 6%, and the method also gives a positive reaction with theobromine.

In addition to the various gravimetric and colorimetric methods for estimating phenobarbital there have been several volumetric methods proposed which give very good results when applied to phenobarbital alone. Among the most important of these methods is the acidimetric method of Babick (7); the turbidimetric method of Budde (8), and Budde's turbidimetric method as modified by Kalinowski (9). The two last-named methods are based on the fact that silver nitrate reacts with phenobarbital in sodium hydroxide solution to form insoluble silver phenobarbital which is extremely weakly disassociated so that the remainder of the sodium hydroxide solution causes a separation of the silver oxide only gradually and incompletely.

Theobromine.—Like phenobarbital, theobromine may be determined in its simple preparations and compounds by the gravimetric method; that is, by either extracting or precipitating the alkaloid and weighing the extract or precipitate as theobromine. An example of this general method is the U. S. P. XI method for the determination of theobromine in Theobromine with Sodium Salicylate (10). In this general method a correction factor must be used because of the solubility of the theobromine. The various gravimetric methods usually give inconsistent results in the hands of different operators probably because of the slight variations in technique; therefore, various correction factors have been proposed.

For many years the most reliable volumetric method for the determination of theobromine was the iodometric method of Emery and Spencer (11). This method depends upon the precipitation of theobromine tetraiodide by the use of an excess of iodine and the determination of the excess of iodine by the use of a standard solution of thiosulfate. The method is more or less unreliable since it gives inconsistent results from one day to another and in the hands of different operators. To overcome these difficulties there

have been various modifications of the method of Emery and Spencer (12, 13, 14, 15, 16). These various modifications depend upon the same principle but vary the method and conditions of the precipitation of the tetraiodide and none of the modifications are very superior to the original method.

The author has found that the most reliable method for the determination of theobromine is the method of Boie (17). This method is much less time-consuming and consistently more accurate than the method of Emery and Spencer or its various modifications. This fact has been confirmed by several reports in the literature including Jorgensen (18) and Van Giffen (19) together with the fact that it has been recently approved as one of the tentative methods of the A. O. A. C. (20).

Briefly this method is based on the fact that silver nitrate reacts with theobromine to form silver theobromine and liberating nitric acid according to the following reaction:

$$C_7H_8N_4O_2 + AgNO_3 \rightarrow AgC_7H_7N_4O_2 + HNO_3$$

The nitric acid thus liberated is titrated with standard alkali. This method for the determination of theobromine has been successfully applied not only to theobromine alkaloid but to many mixtures and salts of theobromine.

EXPERIMENTAL

The usual procedures for the determination of two or more organic drugs of this nature involve the separation of the two or more components of the mixture by the use of a suitable immiscible solvent or mixture of such solvents, evaporating the solvent and determining the amount of extracted material by weight. In combinations of the Theocalcin or theobromine and phenobarbital type, this general procedure is more or less impractical, especially for the determination of phenobarbital, because of the small amount of phenobarbital in relation to the other components. The combinations on the market usually contain 5 grains of theobromine or a corresponding larger amount of a theobromine salt with either $^1/_4$ or $^1/_2$ grain of phenobarbital. Again, this general procedure could not be applied to Theocalcin-phenobarbital mixtures since the phenobarbital must be extracted from an acid solution and any solvent that might be selected to extract the phenobarbital from an acid solution of the mixture would also extract the liberated salicylic acid. Therefore, some other method had to be resorted to.

Experiments proved that salicylic acid does not interfere with the turbidimetric methods of Budde (8) and Kalinowski (9) for the determination of phenobarbital, so it was thought that perhaps this method or some modification of it might serve as a basis for the determination of phenobarbital in mixtures with theobromine calcium salicylate.

After reviewing the various methods outlined above for the determination of theobromine in various mixtures and compounds it was thought that perhaps Boie's acidimetric method (17) offered the best possibilities for adaptation to mixtures of the Theocalcin-phenobarbital type if the active components of the mixture could be separated. For this purpose, as can be seen from Table I, ether offers the best possibilities since phenobarbital is more soluble and theobromine less soluble in ether than in any one of the other solvents or combinations of these solvents investigated. The use of the alcohol-chloroform-ether mixture used in the N. F. method for extracting phenobarbital in various preparations is impractical because of the relatively greater solubility of theobromine. Although ether will extract a very slight amount of the theobromine along with the phenobarbital, the amount is insignificant and does not interfere with the methods outlined below. The ether, however, should be free from alcohol—this is very important.

Table I.—The Solubility of Theobromine and Phenobarbital in Seven Solvents (Compiled from Various Sources)

Note: Solubility given in Gm. per 100 cc. of Solvent at Room Temperature.

Solvent	Theobromine	Phenobarbital
Alcohol	0.040	12.500
Benzene	0.005	0.143
Carbon tetrachloride	0.020	Very slightly sol.
Chloroform	0.060	2.500
Ether	0.003	7.692
Petroleum ether	0.000	0.005
Water	0.050	0.100

METHODS

I. Mixtures Containing Phenobarbital and a Theobromine Salt Such as Theocalcin.—After numerous trials the following method for the analysis of Theocalcin-phenobarbital combinations was adopted:

Theobromine.—Weigh a sample of the mixture corresponding to approximately 15 grains of Theocalcin and transfer the sample to a 250-cc. beaker. Add 50 cc. of water followed by about 50 cc. of approximately 0.1*N* sulfuric acid solution. Boil slowly until a solution results or, if tablet diluents are present, until a homogeneous opalescent mixture results—about two or three minutes. Cool the solution to about 40° C. and then transfer it to a 250-cc. separatory funnel, rinsing the beaker with several small portions of boiling water. To the luke-

warm solution and washings in the separatory funnel add 50 cc. of ether, which is free from alcohol, and shake vigorously. Collect the lower or aqueous layer in another 250-cc. separatory funnel and filter the ether layer through filter paper, discarding the filtrate. Continue to shake out the aqueous solution and washings with three successive portions of 25 cc. of alcohol-free ether, shaking at least two minutes after each addition and each time collecting the aqueous layer in the other separatory funnel and filtering the ether layer. Finally collect the aqueous solution in a 500-cc. wide-mouth Erlenmeyer flask washing each of the two separatory funnels with several small portions of boiling water to remove any adhering material which might be present. Collect the washings in the Erlenmeyer flask and finally place the filter paper from the ether washings into the Erlenmeyer flask along with the extracted aqueous solution and washings. Boil the solution on a hot-plate until the ether has been removed from the solution and then cool to about 40° C. Add $1^1/_2$ cc. of Phenol Red solution (0.02%) and make the solution distinctly alkaline with a slight excess (1–2 cc.) of 0.1*N* sodium hydroxide solution. Then adjust the solution by the careful addition, drop by drop, of 0.1*N* sulfuric acid solution until the bluish red color is discharged and the first distinctly yellow color is produced. Then add about 40 cc. of approximately 0.1*N* silver nitrate solution and titrate the liberated nitric acid slowly with 0.1*N* sodium hydroxide solution until the solution just acquires a bluish red color. Each cc. of 0.1*N* sodium hydroxide solution consumed in the neutralization of the nitric acid (that is, after the addition of the silver nitrate solution) is equivalent to 0.01801 Gm. of theobromine.

Phenobarbital.—Accurately weigh a sample of the mixture corresponding to about four grains of phenobarbital. Transfer the sample to a 250-cc. beaker and add 150 cc. of 2*N* sulfuric acid. Boil slowly until the sample is in solution, or if tablet diluents are present, until a homogeneous, opalescent mixture results, usually about three minutes. Cool the mixture to about 40° C. and transfer it to a 400-cc. separatory funnel, rinsing the beaker with several small portions of boiling water. To the lukewarm solution and washings in the separatory funnel add 50 cc. of alcohol-free ether and shake for about three minutes. Collect the ether layer in a 250-cc. Erlenmeyer flask, filtering through a pledget of cotton. Then continue to shake out the aqueous solution and washings with four successive portions of 25 cc. of alcohol-free ether, shaking at least two minutes after each addition; each time the ether layer is filtered through the pledget of cotton and collected in the Erlenmeyer flask. Two separatory funnels are used alternately as in the case with the theobromine determination. Finally wash the stems of the separatory funnels with a little ether and wash the cotton and funnel with several small portions of ether, collecting the washings in the Erlenmeyer flask. The aqueous layer is discarded and the combined ether extract and washings are heated on a water bath to distil off the solvent. The last few cc.'s of ether are allowed to evaporate at room temperature or not over 40° C. The residue is dissolved in exactly 100 cc. of a solvent composed of 50 cc. of ethyl alcohol, 10 cc. of water and 40 cc. of 1.0*N* sodium hydroxide solution. (The components of this solvent must be mixed and cooled to room temperature before adding to the phenobarbital extract.) After the phenobarbital is dissolved, the solution is filtered through a quantitative grade of filter paper and the first ten cc. of the filtrate are discarded. Transfer exactly 75 cc. of the filtrate, which must be perfectly clear, to a 250-cc. Erlenmeyer flask. This aliquot part is equivalent to $^3/_4$ of the original sample or, in this instance, about three grains of phenobarbital. Titrate the clear solution with 0.1*N* silver nitrate solution, using a 10-cc. burette, until the first distinct turbidity is evident and one that persists after thoroughly shaking. The silver nitrate solution must be added very slowly and toward the end-point must be added drop by drop shaking vigorously after the addition of each drop. The immediate turbidity caused by the addition of each drop must be cleared by shaking before the addition of the next drop, until the final end-point is reached which is characterized by the persistent turbidity of the solution even after vigorous shaking. The titrated solution at this stage begins to change rapidly to a brownish black color due to the separation of the silver oxide. Each cc. of 0.1*N* silver nitrate solution is equivalent to 0.023212 Gm. of phenobarbital.

The results of a typical series of determinations of phenobarbital and theobromine in Theocalcin mixtures are shown in Table II. It will be noted that the determinations were made in triplicate by two different operators. The known samples were carefully made to contain the same ratio of Theocalcin and phenobarbital as the commercial product; namely, $7^1/_2$ grains of Theocalcin to $^1/_4$ grain of phenobarbital.

Table II.—Results of Analyses of Theocalcin-Phenobarbital Mixtures

		Theobromine in Theocalcin			Phenobarbital		
Operator	Sample	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery
A	1	7.19	7.18	99.86	3.000	2.988	99.60
	2	7.19	7.18	99.86	3.000	3.026	100.87
	3	7.19	7.17	99.72	3.000	2.981	99.37
B	1	7.20	7.19	99.86	3.000	2.967	98.90
	2	7.20	7.19	99.86	3.000	2.939	97.97
	3	7.20	7.19	99.86	3.000	2.991	99.68

II. Mixtures of Theobromine and Phenobarbital.—Although Method I is recommended for most mixtures of theobromine and phenobarbital a somewhat simplified method may be employed for separating the components of simple mixtures—namely, by the use of a continuous extraction apparatus with alcohol-free ether as a solvent. The modified methods follow below.

Theobromine.—Accurately weigh a sample of the mixture corresponding to about ten grains of theobromine and transfer it to a small paper extraction thimble. Connect to a simple continuous extraction apparatus of the rubber extraction type using about 75 cc. of alcohol-free ether as a solvent. Extract for about thirty minutes and discard the ether containing the phenobarbital. Place the paper extraction thimble containing the theobromine in a 500-cc. wide-mouth Erlenmeyer flask which contains 150 cc. of water and 25 cc. of approximately 0.1*N* sulfuric acid solution. Allow the paper thimble to disintegrate in this solution or carefully tear it apart using a stirring rod. Boil the solution vigorously for one to two minutes and then cool to about 40° C. Add 1½ cc. of Phenol-red solution (0.02%) and make the solution distinctly alkaline with a slight excess (1–2 cc.) of 0.1*N* sodium hydroxide solution. Then adjust the solution by the careful addition, drop by drop, of 0.1*N* sulfuric acid solution until the blusih red color is discharged and the first distinctly yellow color is produced. Then add about 50 cc. of approximately 0.1*N* silver nitrate solution and titrate the liberated nitric acid slowly with 0.1*N* sodium hydroxide until the solution just acquires a bluish red color. Each cc. of 0.1*N* sodium hydroxide solution consumed in the neutralization of the nitric acid (that is, after the addition of the solution of silver nitrate) is equivalent to 0.01801 Gm. of theobromine.

Phenobarbital.—Accurately weigh a sample of the mixture corresponding to about four grains of phenobarbital, and transfer it to a paper extraction thimble. Connect to a simple extraction apparatus as described above using 100 cc. of alcohol-free ether. Extract for thirty minutes and discard the paper thimble containing the theobromine. The ether, which contains the phenobarbital, is distilled off on a water bath until 2 to 3 cc. remain. The last trace of ether is allowed to evaporate at room temperature or not over 40° C. The residue is dissolved in exactly 100 cc. of a solvent composed of 45 cc. of ethyl alcohol, 30 cc. of water and 25 cc. of 1.0*N* sodium hydroxide solution. (The components of this solvent must be mixed and cooled to room temperature before adding to the phenobarbital extract.) After the phenobarbital is dissolved, the solution is filtered through a quantitative grade of filter paper, discarding the first ten cc. of the filtrate. Transfer exactly 75 cc. of the filtrate, which must be perfectly clear, to a 250-cc. Erlenmeyer flask; this aliquot part is equivalent to ¾ of the original sample or, in this instance, about three grains of phenobarbital. Titrate the clear solution with 0.1*N* silver nitrate solution until the first distinct and persistent turbidity results, using the precautions as outlined for the determination of phenobarbital in mixtures with Theocalcin which is outlined above. Each cc. of 0.1*N* silver nitrate solution is equivalent to 0.023212 Gm. of phenobarbital.

The results of a typical series of determinations of theobromine and phenobarbital in mixtures are shown in Table III. The determinations were made in triplicate by two different operators on samples containing two different ratios of theobromine and phenobarbital; one series of samples contained a ratio of 5 grains of theobromine to ¼ grain of phenobarbital whereas the other series contained a ratio of 5 grains of theobromine to ½ grain of phenobarbital.

Instead of using a solvent composed of ethyl alcohol, sodium hydroxide solution and water for dissolving the extracted phenobarbital, it is possible to substitute acetone for the ethyl alcohol. In this event the solvent should be composed of 25 cc. of 1.0*N* sodium hydroxide solution, 35 cc. of acetone and 40 cc. of distilled water per hundred cc. of solvent in the case of theobromine-phenobarbital mixtures. An attempt was made to use a dilute (3½%) solution of sodium carbonate for dissolving the extracted phenobarbital but the results were inconsistent for some unexplained reason, perhaps because of the indefinite end-point.

Because of the small amount of phenobarbital in the sample and the relatively large phenobarbital equivalent when using 0.1*N* silver nitrate solution,

Table III.—Results of Analyses of Theobromine-Phenobarbital Mixtures

		Theobromine			Phenobarbital		
Operator	Sample	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery
A	1	10.00	9.970	99.70	3.000	2.964	98.80
	2	10.00	9.984	99.84	3.000	2.964	98.80
	3	10.00	9.960	99.60	3 000	3.019	100.63
	4	10.00	9.900	99.00	6.000	5.962	99.37
	5	10.00	10.000	100.00	6.000	6.018	100.30
	6	10.00	10.060	100.60	6.000	5.982	99.70
B	1	10.00	9.986	99.86	3.000	3.008	100.27
	2	10.00	10.000	100.00	3.000	3.002	100.07
	3	10.00	9.986	99.86	3.000	2.973	99.10
	4	10.00	10.018	100.18	6.000	5.926	98.77
	5	10.00	10.018	100.18	6.000	5.965	99.42
	6	10.00	10.028	100.28	6.000	5.990	99.83

an attempt was made to use 0.05*N* silver nitrate solution. Again the end-point was rather indefinite and therefore the results were inconsistent.

The common tablet diluents, such as starch and talcum, were used in making up the experimental samples and they offered no special difficulties. However, stearic acid and its salts were found to interfere with the phenobarbital determination due to reaction with silver nitrate.

The outlined methods for the determination of theobromine in mixtures with phenobarbital are very accurate as shown by the results recorded in Tables II and III but there are certain precautions which should be observed: the titration must be carried out at a slow rate, about two to three cc. per minute during the beginning of the titration and drop by drop toward the end-point; carbon dioxide must be removed from the solution by boiling; the volumetric solutions should be standardized using phenol red as an indicator; and finally the operator should acquire experience in detecting the end-point by running several samples of pure theobromine alkaloid.

Although the method outlined for the determination of phenobarbital in mixtures is slightly long and requires some skill, it gives reasonably good results as shown by Tables II and III. Again there are certain precautions which must be observed: it is important that no alcohol be present in the ether employed for the extraction of the phenobarbital; the alkalinity of the solution of extracted phenobarbital at the time of titration should not be varied appreciably from the figures mentioned because the sharpness of the end-point is influenced by this factor; since only a small amount of silver nitrate solution is used in the titration a small burette (usually 10 cc. size) graduated in 0.05 cc. should be used; and most important of all, the experience and technique of the operator are very important. It is absolutely essential that the operator familiarize himself with the method, particularly the end-point, by running several samples of pure phenobarbital. It should be pointed out again that the success of the method depends largely on the skill and experience of the operator.

In conclusion, it might be stated that the outlined methods have been used successfully by this laboratory for a period of two years and have also been successfully applied to mixtures of other manufacturers.

SUMMARY

1. The literature does not show a method for the determination of theobromine, or theobromine salts, and phenobarbital in mixtures, but the literature is briefly reviewed for methods of determining theobromine and phenobarbital individually.

2. A method is described for the determination of theobromine in mixtures with phenobarbital which consists essentially of separating or removing the phenobarbital, forming silver theobromine and liberating nitric acid quantitatively by the use of a silver nitrate solution. The nitric acid is then titrated with standard alkali.

3. A method is described for the determination of phenobarbital in mixtures with theobromine or theobromine salts which consists essentially of first separating the phenobrabital and using a silver nitrate solution to form insoluble silver phenobarbital which in a dilute sodium hydroxide solution is extremely weakly disassociated so that the remainder of the sodium hydroxide solution causes a separation of silver oxide gradually and incompletely.

4. The solubilities of both theobromine and phenobarbital in various solvents are tabulated.

5. The results of a series of determinations are tabulated to show the dependability of the methods. Samples were used which contained theobromine or Theocalcin and phenobarbital in the ratios normally found. Eighteen determinations of phenobarbital in various samples averaged 99.75% recovery and eighteen determinations of theobromine in various samples averaged 99.90% recovery.

ACKNOWLEDGMENT

The author wishes to express his indebtedness to Mr. R. O. Hauck for his suggestions and assistance in carrying out this work.

REFERENCES

(1) The National Formulary, 6th Edition, Mack Printing Company, Easton, Pa. (1936), page 381.

(2) Montignie, E., *Bull. soc. chim.* (5), 2 (1935), 373–375.

(3) Bougault and Guillou, *Compt. rend.*, 193 (1931), 463–466.

(4) Fleury, Paul, *Bull. soc. chim.*, 37 (1925), 1656–1668.

(5) Zwikker, *Pharm. Weekblad.*, 68 (1931), 975.

(6) Dille & Koppanyi, *Jour. A. Ph. A.*, 23 (1934), 1079.

(7) Babick, S., *Pharm. Monatsch.*, 17 (1936), 87.

(8) Budde, Hans, *Apoth. Ztg.*, 49 (1934), 295.

(9) Kalinowski, Kazimierzi, *Wiadomości Farm.*, 62 (1935), 633–635 & 649–657.

(10) Pharmacopœia of the United States, XI Revision, Mack Printing Co., Easton, Pa. (1936), page 383.

(11) Emery, W. O., and Spencer, G. C., *Ind. Eng. Chem.*, 10, No. 8 (1918), 605–606.
(12) Matthes and Schutz, *Pharm. Ztg.*, 75 (1930), 42–44.
(13) Saiko, Berta-Pittner, *Pharm. Monatsch.*, 11 (1930), 73.
(14) Miko, Gyula, *Arch. pharm.*, 270 (1932), 163–167.
(15) Van Breukelevean, M., *Chem. Weekblad*, 24 (1927), 206–207.
(16) Boie, Heinrich, and Linder, Alois, *Pharm. Ztg.*, 74 (1929), 418–420.
(17) Boie, Heinrich, *Ibid.*, 75 (1930), 968–969.
(18) Jorgensen, P. S., *Jour. A. O. A. C.*, 21 (1938), 555–557.
(19) Van Giffen, H. J., *Pharm. Weekblad*, 69 (1932), 1321–1325.
(20) Methods of Analysis of A. O. A. C., 5th Edition (1940), page 593.

Note on the Behavior of Sulfonamides in the Cobalt Color Tests for Barbiturates

*By Theodore Koppanyi, Melvin W. Green and Charles R. Linegar**

Recently, samples of sulfathiazole contaminated with phenobarbital were found on the open market. In connection with this finding, Dr. Knudson of the Albany Medical College and others called our attention to the fact that the Koppanyi test for barbiturates is also positive to the sulfonamides. In the fear that some unadulterated sulfonamide preparation might be rejected, unfairly, on the basis of this color test, we have investigated the behavior of sulfathiazole, sulfanilamide and sulfapyridine with reference to the cobalt color tests. We are concerned here with two different aspects of the same problem: (1) the differentiation of barbiturates from sulfonamides in pharmaceutical analytical work, and (2) the possible interference of sulfonamides, in the urine and other body fluids, in the diagnosis of barbiturate poisoning.

EXPERIMENTAL

1. Dille and Koppanyi (1) have shown that the cobalt acetate-isopropylamine test may be used to assay pharmaceutical preparations containing barbiturates. In that procedure the alkaline preparation is dissolved in water, acidulated and then shaken out with at least ten volumes of chloroform.

The same procedure was followed using prepared mixtures containing known amounts of phenobarbital and different sulfonamides.

Table I indicates that the presence of sulfonamides does not appreciably interfere with the pharmaceutical assay of barbiturate preparations provided the Dille-Koppanyi procedure is strictly adhered to. The sample containing 100 mg. of sulfonamides with no barbiturates yields less color, in the colorimetric procedure, than does 2.0 mg. of phenobarbital. The presence of phenobarbital or any barbiturate in admixture with any suspected sulfonamide preparation can thus be detected by the following procedure. Weigh out a 100-mg. sample of the suspected sulfonamide, dissolve in 20 cc. of water, acidulate and shake out with 20 volumes of chloroform. Simultaneously, subject a 100-mg. sample of the pure sulfonamide to the same procedure. Evaporate the chloroform extract to dryness and take up the residue in a convenient, measured volume of chloroform. Compare each of these chloroform solutions with suitable standard solutions (0.02 to 0.08 per cent in chloroform) of the barbiturate in question. If the suspected sulfonamide produces a more intense color than the pure sulfonamide itself, the preparation is contaminated. And, moreover, the degree of adulteration can be

Table I.—Recovery of Phenobarbital from Phenobarbital-Sulfonamide Mixtures as Determined by the Cobalt-Isopropylamine Method

Sulfonamides Used	Amount of Sulfonamide in Sample, Mg.	Amount of Phenobarbital in Sample, Mg.	Color Comparison in Terms of Pure Phenobarbital as a Standard, Mg.	Percentage Phenobarbital Recovery, Per Cent
Sulfathiazole	100	0.0	<2.0–	.....
Sulfathiazole	95	5.0	4.6– 5.7	91.2–114
Sulfathiazole	90	10.0	9.2–10.4	92.4–104
Sulfathiazole	50	50.0	54.0–56.7	108.0–113.4
Sulfapyridine	100	0.0	<2.0–	.....
Sulfapyridine	50	50.0	54.5	109.0
Sulfapyridine[a]	50	50.0	58.0	116.0

[a] Determination made in the chloroform solution directly.

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readily determined. Since some sulfonamides, particularly sulfathiazole, in chloroform and chloroform-alcoholic solutions give a strong color with the cobalt-isopropylamine reagent, it should be reiterated that the assay must be carried out strictly as directed and not by attempting to dissolve the samples directly in chloroform.

2. It is not likely that toxic amounts of barbiturate would be taken by patients receiving energetic sulfonamide medication, nor is it likely that sulfonamides alone would be taken in sufficiently large doses to produce acute collapse. Nevertheless, the possibility must be faced that an occasional case will present itself to a hospital laboratory where diagnosis of acute sulfonamide poisoning might be complicated by a previous barbiturate medication. In such cases the urine and blood, when properly treated, may give a positive cobalt color test due to the presence of either sulfonamide or barbiturate. Even in these rare cases it should not be difficult to demonstrate the presence or absence of barbiturates.

In saturated chloroform-alcoholic solutions all three of the sulfonamides studied give positive reactions with the barium, lithium and isopropylamine tests (Koppanyi, *et al.* (2)), but positive barium and lithium tests are not obtainable if the sulfonamides are extracted from 2.0 per cent aqueous media with chloroform.

Since the maximum concentration of free sulfonamide to be expected in the urine or other tissue fluids under the most extreme conditions would not exceed 2.0 per cent, it follows that urines from patients receiving sulfonamides alone would be negative with the barium and lithium tests, and positive only with the isopropylamine test. Using the shake-out procedure, if the barium and lithium tests are positive, this indicates that barbiturates are present in the urine in addition to sulfonamides.

It is, therefore, easy to diagnose barbiturate poisoning and distinguish it from sulfonamide intoxication. The barium and lithium tests, which are not only qualitative but also approximately quantitative, should be performed first on body fluids of suspected cases, and only if these tests are positive should one proceed with the more quantitative isopropylamine tests.

Recently, all three tests have been performed on a number of pathological urines of treated and untreated patients. In no case was a positive test obtained unless the patient had received barbiturate medication. These findings will be reported in the near future.

REFERENCES

(1) Dille, J. M., and Koppanyi, T., JOUR. A. PH. A., 23 (1934), 1079.

(2) Koppanyi, T., Dille, J. M., Murphy, W. S. and Krop, S., *Ibid.*, 23 (1934), 1074.

Umbellatine from *Berberis insignis*, Hook. f.

*By R. Chatterjee**

It is well known that alkaloids occur in the species of *Berberis*, family *Berberidaceæ*. Table I is a résumé of the investigations recorded in literature.

The results show that several other alkaloids together with berberine occur in the species *Berberes*. Though several European varieties have been worked up, few references are found for Himalayan *Berberes*. Chopra and others (7) in their study of the medicinal plants which grow in the Himalayas mention the total alkaloid contents of *B. asiatica*, as a mixture of berberine and oxyacanthine. Because of their yellow stem, the *Berberes* are called in Sanscrit *Daruharidra* (yellow wood), an extract of which was used by the Hindus, Greeks and Arabs for medicinal purposes. The extract was commonly used in India as a remedy against malaria and diarrhea. It was also applied to any external inflammation and in the treatment of oriental sores (7).

Several varieties of Himalayan *Berberres* have been referred to by Chopra (13) as berberine-containing plants. *B. umbellata* Wall., growing in the Himalayas, at an altitude of 9000–11,000 ft. (14), showed the absence of berberine and yielded a new alkaloid, called umbellatine (12). It was suspected that other Himalayan *Berberes* also may not contain berberine at all. The present paper deals with the isolation and identification of umbellatine, in another species of Himalayan *Berberes*, *B. insignis*, Hook. f., which grows at an altitude of 7000–10,000 ft., in the humid forests of the Eastern Himalayas from Nepal to Bhotan (14).

I could procure only stem-barks for the extraction of the alkaloid. However, a gravimetric assay showed the presence of the largest amount of the alkaloid in the roots.

It was in the month of May that I obtained the stem-barks of *B. insignis*, Hook. f. grown at a place near Darjeeling, 6500 ft. above sea level. I could find only one

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Table I.—Alkaloids of *Berberis* Species

Species	Alkaloid	Empirical Formula	Melting Point, ° C.	References
B. vulgaris, L.	Berberine	$C_{20}H_{19}O_5N$	144	(1)
	Berbamine	$C_{18}H_{19}O_3N$	156	
	Oxyacanthine	$C_{19}H_{21}O_3N$	208–214 (Hesse)	
	Columbamine	$C_{21}H_{22}O_5NI$ (the iodide)	224 (the iodide)	
	Palmatine	$C_{21}H_{22}O_4NI$ (the iodide)	240 (the iodide)	
	Jatrorrhizine	$C_{20}H_{20}O_5NI$ (the iodide)	208–210 (the iodide)	
	Unnamed base	$C_{19}H_{22}ON_2$	256 (the hydrochloride)	
	Berberrubine			
B. nervosa, Pursh.	Berberine			(2)
B. glauca, D. C.	Berberine			(3)
B. aquifolium	Berberine			(4)
	Berbamine			
	Oxyacanthine			
B. buxifolia, Laun.	Berberine			(5)
B. aetnensis	Berberine			(6)
B. asiatica	Berberine			(7)
	Oxyacanthine			
B. thunbergii	Berberine			
D. C. var *Maximowiczii*	Oxyberberine	$C_{20}H_{17}O_5N$	199–201	(8)
	Jatrorrhizine			
	Columbamine			
	Berbamine			
	Oxyacanthine		217 (Kondo and Tomita)	
	Shobakunine		204–206 (the iodide)	
	Tetrahydroshobakunine	$C_{20}H_{23}O_4N$	140	
B. Darwinii, Hook.	Berberine			(9)
B. heteropoda, Schrenk.	Berberine			(10)
	Berbamine			
	Oxyacanthine			
	Jatrorrhizine			
	Columbamine			
	Palmatine			
B. laurina, *Billb.* (Thunb)	Berberine			
	Hydrastine	$C_{21}H_{21}O_6N$	132	(11)
B. umbellata, Wall.	Umbellatine	$C_{21}H_{21}O_4N$	206–207 (decomp.)	(12)

alkaloid, whose analytical data and properties and derivatives showed it to be identical with the umbellatine found by me in *B. umbellata*, Wall (12).

Table II.—The Total Alkaloid in *B. insignis*, Hook. f.

Stem-wood	0.38 per cent
Stem-bark	1.52 per cent
Whole root	2.50 per cent

EXPERIMENTAL

Preliminary Test for Alkaloids.—About 10 Gm. of powdered stem-barks were extracted with hot alcohol. The residue from the alcoholic extract was treated with water, filtered from resinous matter and then treated with dilute hydrochloric acid. The solution was tested with alkaloidal reagents, such as Mayer's reagent, picric acid and Wagner's reagent. The tests were all positive.

Systematic Examinations.—Fifty Gm. of powdered stem-barks were extracted in a glass Soxhlet successively with petroleum ether (b. p. 40–60° C.), ether, chloroform and absolute alcohol.

Isolation of an Alkaloid and Its Purification.—For isolation of an alkaloid a quantity of 1 Kg. of powdered air-dried stem-bark was percolated exhaustively with 98% ethyl alcohol. The alcohol of the extract was removed and the residue treated with 500 cc. of water and filtered to get rid of resinous matter. The filtrate was treated with hydrochloric acid to give a 1% solution of the aqueous extract, and allowed to stand for 2 hours. The hydrochloride of the alkaloid gradually crystallized out and was filtered off.

Table III.—Results of the Extraction by Different Solvents

Extract	Amount, Per Cent	Nature
Petroleum ether	3.5	A green sticky mass consisting mainly of chlorophyll and waxy matter
Ether	0.9	Waxy matter
Chloroform	3.3	A brownish sticky mass giving very faint reactions for alkaloids
Alcohol	27.6	A brownish sticky mass giving very strong reactions for alkaloids

The crude hydrochloride was purified by crystallizing twice from hot water and finally from hot alcohol. The yellow silky crystals of the pure hydrochloride were then dissolved in hot water and a

dilute solution of sodium carbonate added until alkaline. The yellow color of the solution changed to deep brown and on cooling yellow silky needles of the free alkaloid were obtained. They were purified by repeated crystallizations from hot water and finally from hot alcohol. (Yield, 10 Gm.)

The alkaloid melted at 205–207° C. (decomp.). Repeated crystallizations of the alkaloid did not change its melting point. The results of combustion analyses conform to the empirical formula, $C_{21}H_{21}O_8N$.

Analysis.—Calculated for $C_{21}H_{21}O_8N$, $^1/_2H_2O$: C, 59.4; H, 5.2; N, 3.3. Found: C, 59.0; H, 5.3; N, 3.3.

Umbellatine mixed with the alkaloid did not depress the melting point of the latter. Mixed melting point of nitrosoumbellatine and nitroso derivative of the alkaloid remained unchanged at 265–267° C. (decomp.). The amount of platinum in the chloroplatinate was found to be 15.63%, whereas platinum present in umbellatine chloroplatinate was determined as 15.71% (12). Calculated for $(C_{21}H_{21}O_8N,HCl)_2PtCl_4$: Pt, 15.72%. The coincidence of these results led me to conclude that umbellatine is present in *B. insignis*, Hook. f.

Umbellatine Sulfate.—Umbellatine sulfate is prepared by the usual method. Samples obtained from both sources melt at 274° C. (decomp.).

Umbellatine Picrate.—On treating a solution of umbellatine in alcohol, with an alcoholic solution of picric acid, the picrate was precipitated at once. The picrate was crystallized from hot alcohol as brown feathery needles. It melts at 232° C. (decomp.).

Analysis.—Calculated for $C_{27}H_{24}N_4O_{15}$: N, 8.7; Found: N, 8.9.

SUMMARY

1. Berberine and associated alkaloids were found to be present in many species of *Berberis*. Himalayan *Berberes*, however, showed the absence of berberine and yielded a different alkaloid.

2. One of the Himalayan *Berberes*, *B. insignis*, Hook. f., contains 1.0% of total alkaloid.

3. An alkaloid has been isolated and is found to be identical with umbellatine, $C_{21}H_{21}O_8N$, the alkaloid of B. umbellata, Wall., a Himalayan *Berberis*.

REFERENCES

(1) Buchner, *Ann.*, 24 (1835), 228; Polex, *Arch. Pharm.*, 6, II (1836), 265; Wacker, *Vjschr. prkt. Pharm.*, 10 (1861), 545; Hesse, *Ber.*, 19 (1886), 3190; Schulz, JOUR. A. PH. A., 15 (1926), 33; Späth and Kolbe, *Ber.*, 58 (1925), 2280; Späth and Polgar, *Monatsh.*, 52 (1929), 117; Schuiz and von Bruchhausen, *Arch. Pharm.*, 267 (1929), 617.

(2) Neppach, *Amer. J. Pharm.*, (1878), 373.

(3) Schilbach, see Wehmer, "Die Pflanzenstoffe," 1929.

(4) Parsons, *Pharm. J.*, 13, III (1882–1883), 46; Stübbe and Rüdel, *Arch. Pharm.*, 229 (1891), 631.

(5) Arata, *Rep. Pharm.* (1892), 45.

(6) Perkin, A. G., *J. Chem. Soc.*, 71 (1897), 1194.

(7) Chopra, Ghosh and Ratnagiriswaran, *Indian J. Med. Research*, 16 (1929), 776.

(8) Kondo and Tomita, *Arch. Pharm.*, 268 (1930), 549.

(9) Cromwell, *Biochem. J.*, 27 (1933), 860.

(10) Orekhov, *Arch. Pharm.*, 271 (1933), 323.

(11) Gurguel, Costa, da Silva, *Bull. assoc. Brasil. Pharm.*, 15 (1934), 11.

(12) Chatterjee, R., *J. Indian Chem. Soc.*, 17 (1940), 289.

(13) Chopra, R. N., "Indigenous Drugs of India," 1933, page 291.

(14) Hooker, J., "Flora of British India," Vol. I, page 110.

The Stabilization of Liquid Preparations Containing Pepsin*

By C. J. Klemme† and C. L. Boswell‡

INTRODUCTORY

The present work is a continuation of the study of liquid preparations containing pepsin for the purpose of stabilizing the activity of peptic systems. Heretofore, as mentioned by Klemme and Worrell (1), stability studies of pepsin preparations have been inconclusive, due primarily to the lack of a satisfactory method of assay. Klemme and Worrell had this objective in mind, namely, to develop a procedure which could be employed in the accurate assay of pepsin preparations for activity. In the experiments conducted in this research their method of assay has been used entirely.

In the past few years several enzymes have been isolated in pure crystalline form and their chemical nature studied. Pepsin, the enzyme with which we are primarily con-

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cerned, possesses the physical and chemical properties of an albumin, a typical primary protein, and its unusual proteolytic activity is generally associated with the particular arrangement of the constituent amino acids in the pepsin molecule (Northrop (2, 3)). Northrop (4) indicates that a major source of inactivation in the case of pepsin is due to denaturation of the enzyme protein.

Many factors, such as hydrogen ion concentration, temperature, purity of the enzyme, concentration of the enzyme, presence of added electrolytes and other substances, as well as time of exposure to these conditions, affect the activity of the particular enzyme in solution.

In attacking the problem of stabilizing the activity of liquid preparations containing pepsin, it was deemed fundamental that a study first be made of the influence of individual factors or variables on the maintenance of peptic activity over an extended period of time. The factors which have received special consideration include temperature, p_H, antioxidants (maleic acid, hydroquinone, resorcinol), preservatives (glycerol, alcohol, hexylresorcinol, "Merthiolate"), protective agents (acacia), low concentrations of amino acids (tyrosine), agitation and storage under nitrogen.

A number of preparations were made at suitable intervals, the initial ones having a single variable factor, whereas later ones possessed combinations of agents that seemed desirable as a result of a study of the stability of previous samples. All of the solutions were assayed at regular intervals throughout the duration of the study.

EXPERIMENTAL

Armour's U. S. P. Pepsin was used in making all of the preparations for stability tests, and Merck's Casein (according to Hammarsten) was employed as the substrate for determining the peptic activity.

In general, the preparations were made by dissolving the pepsin in distilled water (or a buffer solution prepared from $0.5M$ potassium acid phthalate and $0.5M$ sodium hydroxide), filtering the solution by suction, and then, after washing the filter with a small amount of distilled water, adding the other constituents of the preparation along with enough distilled water or buffer solution to bring the product up to the required volume.

Table I contains a selected list of the preparations studied. Only the initial and final assay figures are

Table I.—A Summary of Assay Results of Pepsin Solutions on Stability Tests

No. Prepn.	Variable Factors	Gm. Pepsin per 100 Cc. Initial	Final	Per Cent Loss in Activity
5	"Merthiolate," 1:5000 p_H 4.5	4.9	5.1/12 mo.	0
27	"Merthiolate," 1:10,000 Under N_2 at 5° C. p_H 4.5	5.1	4.4/18 mo.	14
36	"Merthiolate," 1:10,000 Buffered, p_H 4.0	4.1	3.4/12 mo.	17
6	"Merthiolate," 1:10,000 Buffered, p_H 4.5	5.2	4.5/12 mo.	13
37	"Merthiolate," 1:10,000 Buffered, p_H 4.8	4.0	3.9/12 mo.	3
38	"Merthiolate," 1:10,000 Buffered, p_H 5.2	3.6	3.5/12 mo.	3
39	"Merthiolate," 1:10,000 Buffered, p_H 6.0	3.1	2.8/12 mo.	10
16	Acacia, 1% "Merthiolate," 1:2000 Under N_2 p_H 4.5	4.5	3.1/24 mo.	31
19	Tyrosine, 0.1% "Merthiolate," 1:2000 Under N_2 p_H 4.5	5.3	4.1/24 mo.	23
26	Alcohol, 12% "Merthiolate," 1:10,000 Buffered, p_H 4.5	5.5	4.8/12 mo.	13
8	Alcohol, 8% Under N_2 p_H 4.5	5.2	2.9/24 mo.	46
9	Alcohol, 12% p_H 4.5	4.7	4.5/12 mo.	4
13	Malic Acid, 1% p_H 2.8	5.2	0/8 mo.	100
31	Resorcinol, 1% Alcohol, 4% p_H 4.5	5.0	3.2/18 mo.	36
33	Hexylresorcinol, 1% Alcohol, 4% p_H 4.5	5.0	4.8/18 mo.	4
35	Magnesium Chloride, 0.5% Catechol, 1% Under N_2 p_H 4.5	3.5	2.1/12 mo.	40
22	Glycerol, 20% "Merthiolate," 1:5000 Buffered, p_H 5.0	4.8	4.7/12 mo.	2
10	Glycerol, 50% p_H 4.5	4.7	5.0/18 mo.	0
11	Glycerol, 90% p_H 4.5	4.7	4.8/18 mo.	0
40	Glycerite of Pepsin, N. F. VI p_H 4.2	9.9	10.2/12 mo.	0
42	Glycerol, 50% Aromatic Elixir, 50% p_H 4.4	5.0	4.8/18 mo.	4
44	Elixir of Pepsin, N. F. VI p_H 4.1	3.5	3.6/12 mo.	0
46	Compound Elixir of Pepsin, N. F. VI p_H 4.3	3.2	2.3/12 mo.	28
47	Elixir of Pepsin and Rennin, N. F. VI p_H 4.2	2.4	2.1/12 mo.	13
50[a]	"Aqueous Elixir of Pepsin" p_H 4.1	3.3	3.2/12 mo.	3

[a] Differs from the N. F. VI Elixir of Pepsin in that Aqueous Elixir is substituted for Aromatic Elixir, and that "Merthiolate," 1:5000, is added.

reported in this table. The constituents, other than pepsin, are listed under "Variable Factors." Most of the preparations were stored at room temperature in a closed container. Storage under an atmosphere of nitrogen or at refrigeration temperature was considered to represent a variable factor. Since the hydrogen ion concentration has proved to be of utmost importance, the approximate p_H of each preparation is included.

The limit of error in assaying a preparation from time to time is approximately 6%. If we assume that any preparation retaining 90% or more of the original activity after a year's time is stable for all practical purposes, then it is evident that several of those studied are promising from the standpoint of stability.

Undoubtedly the most important factor pertaining to the stabilization of peptic systems is the hydrogen ion concentration. Our results show that the optimum p_H range for storage of pepsin solutions is from p_H 4.5 to 5.5. A p_H below 4 is definitely unfavorable for the retention of proteolytic activity.

Although solutions containing "Merthiolate" as a preservative retained their activity quite well, a slight reduction of the mercury in the "Merthiolate" was noticeable after a few months of aging.

As a group, all of the preparations containing 20% or more of glycerol retained their original activity throughout the assay period.

Of the N. V. VI preparations, the Elixir of Pepsin and the Glycerite of Pepsin are the most stable. Synder, Goldberg and Gathercoal (5) conclude that the greater stability of the N. F. VI Elixir of Pepsin, as compared to the N. F. V Elixir, results from a better control of the hydrogen ion concentration. The N. F. VI Elixir is buffered at p_H 4.1 approximately, whereas the p_H of the N. F. V product is 2.5. Preparation 13, having a p_H of 2.8, was completely inactive at the end of eight months.

Experiments on the effect of shaking pepsin solutions were also performed. Klemme and Boswell (6) reported that vigorous mechanical agitation for periods of six hours or less produced no discernible losses in peptic activity, whereas sizable losses in proteolytic power occurred after vigorous shaking for over twelve hours. Further experiments in this connection indicated that certain preparations were stable to mild mechanical agitation for a period of one week. Before assaying the preparations following agitation, the froth was allowed to settle completely. Consequently, the statements in the National Formulary regarding the reduction in proteolytic activity of pepsin solutions by excessive shaking or stirring are unnecessary.

A pepsin solution is essentially a colloidal dispersion of a protein, and its stability is determined primarily by the hydrogen ion concentration of the solution and the water of hydration surrounding the pepsin molecules. The stabilizing action of glycerol may be due to its adsorption on the pepsin molecules, forming a film which has a strong attraction for water molecules.

From a careful study of the pepsin solutions on stability tests, we have formulated four preparations which will undergo no noticeable color change or sedimentation, and which will retain their peptic activity over a long period of time without suffering any appreciable loss in activity.

The suggested formulas are as follows:

FORMULA I

Pepsin, U. S. P.	50 Gm.
Citric Acid Crystals	10 Gm.
Exsiccated Sodium Phosphate	14.5 Gm.
Glycerol	200.0 cc.
Aromatic Elixir	200.0 cc.
Distilled Water, a sufficient quantity, To make	1000 cc.

FORMULA II

Pepsin, U. S. P.	35 Gm.
Citric Acid Crystals	10 Gm.
Exsiccated Sodium Phosphate	14.5 Gm.
Distilled Water	300.0 cc.
Glycerol	200.0 cc.
Aromatic Elixir, a sufficient quantity, To make	1000 cc.

Note: The p_H of the above preparation is approximately 5, whereas the N. F. VI Elixir of Pepsin has a p_H slightly over 4.

FORMULA III

Pepsin, U. S. P.	100 Gm.
Glycerol	500 cc.
Distilled Water, a sufficient quantity, To make	1000 cc.

Note: The above product differs from the N. F. VI Glycerite of Pepsin in that the diluted hydrochloric acid is omitted. The p_H of the above product is approximately 4.5.

FORMULA IV

Pepsin, U. S. P.	50 Gm.
Citric Acid Crystals	10 Gm.
Exsiccated Sodium Phosphate	14.5 Gm.
Glycerol	500.0 cc.
Distilled Water, a sufficient quantity, To make	1000 cc.

The method of preparation of the above products is of considerable importance. The suggested procedure is as follows: Dissolve the citric acid and exsiccated sodium phosphate in a sufficient amount of distilled water (about 200 to 300 cc.), add the pepsin and stir until dissolved. (In Formula III, the buffer substances are omitted.) Allow the solution to stand over night in a refrigerator. Filter the solution, preferably by suction, and wash the filter thoroughly with distilled water. To the clear filtrate add the glycerol and the remaining constituents along with enough distilled water to bring the product up to the required volume. Mix thoroughly and place in a closed container.

COMMENTS

In making the above preparations it is not necessary to avoid agitation. The formulas may be varied slightly as long as the p_H is maintained in the neighborhood of 4.5 to 5.5, and as long as 20% or more of glycerol is present. An alcoholic concentration above 15% should be avoided. For best results, the method of preparation should be carried out as directed above. If filtration by suction is not available, filtration by gravity may be employed, being certain that the filter paper is washed thoroughly with distilled water. Finally, the preparations should be assayed for peptic activity before placing in permanent flasks.

BIBLIOGRAPHY

(1) Klemme, C. J., and Worrell, L. F., JOUR. A. PH. A., 29 (1940), 263.
(2) Northrop, J. H., *J. Gen. Physiol.*, 13 (1930), 739.
(3) Northrop, J. H., *Ibid.*, 14, (1931), 713.
(4) Northrop, J. H., *Ibid.*, 16 (1932), 33.
(5) Snyder, R. K., Goldberg, L., and Gathercoal, E. N., JOUR. A. PH. A., 26 (1937), 541.
(6) Klemme, C. J., and Boswell, C. L., *Ibid.*, 29 (1940), 497.

Carcinogenics and Medicine*

By Seward E. Owen†

This report deals with a consideration of the carcinogenic chemicals, their sources, biological activity, chemistry and methods of detection as they relate to medicine.

Certain tars were first shown to be capable of producing cancer in experimental animals by Yamagiwa and Ichikawa (1) in 1913. Since that time much progress has been made in the clarification of the chemical formulas of substances capable of inducing malignant changes in the tissues of experimental animals. Important contributions are those of Bloch and Dreifuss (2) who showed that carcinogenic material in tar was contained in the nitrogen-free, neutral, high-boiling fraction. That the active substances were hydrocarbons was indicated by the work of Kennaway (3). Mayneord (4) first applied the fluorescent spectrum method to the problem. Heiger (5) later noted that many carcinogenics displayed similar fluorescence spectra. The absorption spectrum of 1:2:5:6 dibenzanthracene was first studied by Clar (6) and later by Chalmers (7). These early spectrum studies led the English scientists to try many compounds with similar spectra for possible carcinogenic effects on animals, with the result that 1:2:5:6 dibenzanthracene was found to be a potent cancer-causing agent when applied in solution to the skin of animals. By this technique cancers of the squamous variety were induced. Later Burrows, *et al.* (8), noted that the same agent in contact with connective tissue produced tumors of this tissue. Cook, *et al.* (9), synthesized a large number of similar compounds which were tested biologically. Among these were methylcholanthrene and 1:2 benzpyrene, both highly carcinogenic.

The most important carcinogenic chemicals that may exist in coal tar are 1:2 benzpyrene, 1:2:5:6 dibenzanthracene and possibly methyl cholanthrene. These are now made synthetically for experimental use and may be obtained from the standard chemical houses. Reviews from the chemical standpoint of carcinogenics have been presented by Cook and Kennaway (10) and by Fieser (11). These are rather exhaustive and complete. Many derivatives of the above carcinogens as well as some newer compounds are mentioned in the above papers. Other reviews consider the carcinogenics, sex hormones and sterols, all of which have the phenanthrene nucleus Owen (12) and Dodds (13). In this connection it is of interest to point out that some synthetic carcinogenics are estrogenic and that rather potent estrogenics may be derived from certain closely related, chemically, carcinogenic agents.

The test methods to demonstrate carcinogenic activity are those adopted by the English workers which consist of applying the hydrocarbons at 0.3% in benzene solution to the interscapular region of mice

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† From The Cancer Research Unit, Veterans' Administration, Hines, Illinois.

twice weekly, the applications usually being continued until tumors are noted or for a period of months. The technique utilized in leading American laboratories is that of Shear (14) who injects 5 to 10 milligrams as a solid moistened with a little glycerol. The injections are usually made subcutaneously into mice. A single injection is usually sufficient. The carcinogenic hydrocarbons are considered to be relatively insoluble in water or in aqueous salt solutions and most of them are but sparingly soluble in organic solvents as benzene, alcohol or lipids. As little as 0.4 gamma of 1:2:5:6 dibenzanthracene has caused the appearance of cancer. The use of the higher dosages as 5 to 10 mg. minimizes any variations connected with the dosage factor in analyzing a group of substances for carcinogenicity. Unfortunately, lard and the liquid and filtrable part of lard at 37° C. have been used as a solvent. This, however, is of a variable composition which may undergo changes on heating or on storage. Nearly all of our mice of the C3H strain have developed tumors following the injection of 0.25 mg. of the 1:2:5:6 dibenzanthracene (Owen (15)). Instances of varying susceptibility to the carcinogenics by various strains of mice are noted by Fieser (11). Average induction time of cancer ranges from 13 to 30 weeks, depending upon the route and animals used.

There are no outstanding agents used therapeutically which might contain the carcinogenics other than the tars and the oil of tar which is a distillation product of pine tar. The prepared coal tar of the British Pharmacopœia might be considered to be more dangerous from the carcinogenic viewpoint. The wood tars to date have not been incriminated but their use might well be questioned now that other substances are available Sollman (16). In instances where tar is to be used for therapeutic purposes, and especially if it be coal tar, then resort should be had to the tests for the presence of carcinogenics. The most promising test presented is that of the absorption spectra, as used by Lorenz and Shear (17). Color reactions with *p*-nitro-benzenediazonium chloride are mentioned by Fieser (*loc. cit.*) but it is noted that this test gives questionable results with 1:2:5:6 dibenzanthracene, the 10-methyl and ethyl derivatives of cholanthrene, with 5,10-dimethyl-1,2-benzanthracene and with 3-methyl-1,2-benzanthracene, all of which are carcinogenic. Phenols and amines must be removed previous to the chemical test also as they interfere. The biological assay method is finally the certain method of detection although the time factor is an obstacle here, whether the tar itself or extracts of it are employed. Eventually appropriate organic solvents may be found to extract the carcinogenics from tars for therapeutic uses; at present, however, alcohol, benzene, chloroform and petroleum ether all dissolve one or more of the known carcinogenics from coal tar. It is questionable whether bituminous shale products which are commonly sulfonated before use, contain carcinogenic substances. No researches have been conducted on this question as yet but undoubtedly such studies will be initiated in the future.

SUMMARY

1. A consideration of the chemical carcinogenics is presented with special reference to therapeutic use of agents that might possibly contain these agents.

2. It is suggested that appropriate tests of the medicinal tars and related products be made for the carcinogenics before these substances are recommended for therapeutic use.

3. The available tests for carcinogenics including the chemical, biological and physical (spectra) are discussed as to their efficiency.

Thanks are expressed to Dr. Max Cutler for his aid and interest in the preparation of this paper.

BIBLIOGRAPHY

(1) Yamagiwa, K., and Ichikawa, K., *Mitt. med. Facultat kaiser. Universitat, Tokyo*, 15 (1915–1916), 295–344.

(2) Bloch, B., and Dreifuss, W., *Schweiz. med. Wochschr.*, 2 (1921), 1033–1037.

(3) Kennaway, E. L., *J. Path. Bact.*, 27 (1924), 235–238; *Biochem. J.*, 24 (1930), 497–504; *Brit. Med. J.*, 2 (1925), 1–4.

(4) Mayneord, W. V., quoted by E. C. Dodds in *Lancet*, 226 (1934), 987–992.

(5) Heiger, I., *Biochem. J.*, 24 (1930), 505–511.

(6) Clar, E., *Ber.*, 62 (1929), 350–359.

(7) Chalmers, J. G., *Biochem. J.*, 28 (1934), 1214–1218.

(8) Burrows, H., Heiger, I., and Kennaway, E. L., *Am. J. Cancer*, 16 (1932), 57–67.

(9) Barry, G., Cook, J. W., Haslewood, G. A. D., Hewett, C. L., Heiger, I., and Kennaway, E. L., *Proc. Roy. Soc.* (London) B, 117 (1935), 318–351.

(10) Cook, J. W., and Kennaway, E. L., *Am. J. Cancer*, 33 (1938), 50–97.

(11) Fieser, L. F., *Ibid.*, 34 (1938), 37–124.

(12) Owen, S. E., *Quart. Rev. Biol.*, 12 (1937), 340–347.

(13) Dodds, E. C., *Lancet*, 226 (1934), 931–934 and 987–992.

(14) Shear, M. J., *Am. J. Cancer*, 26 (1936), 322–332.

(15) Owen, S. E., unpublished data.

(16) Sollman, T., "A Manual of Pharmacology," 3rd Edition, page 640, Saunders and Co.

(17) Lorenz, E., and Shear, M. J., *Am. J. Cancer.*, 26 (1936), 333–343.

Book Reviews

Laboratory Exercises in the Chemistry of Medicinal Products, by WALTER H. HARTUNG, Ph.D., Professor of Pharmaceutical Chemistry, University of Maryland, W. TAYLOR SUMERFORD, Ph.D., Professor of Pharmaceutical Chemistry, University of Georgia, and MELVIN F. W. DUNKER, Ph.D., Fellow in Chemistry, Northwestern University. 152 pages, mimeographed by the University of Georgia Press. Price, $2.75.

This laboratory manual is intended for advanced undergraduate and beginning graduate students in pharmaceutical chemistry. The discussion, questions and experiments are designed to develop in the students a capacity to appreciate and understand better the principles on which medicinal and pharmaceutical chemistry are based. Wherever practicable, the experiments are carried out on the semimicro scale. The work is grouped in sixteen chapters headed as follows: Hydrocarbons, Halogenated Hydrocarbons, The Hydroxyl Group, Hydroxyl and Halogen Derivatives, Ethers, The Carboxyl Group, The Carbonyl Group, Nitrogen Compounds, Sulfur Compounds, Organometallic Compounds, Spot Tests, Library Problem, The Chromatograph, Dyes, Enzymes, Glycosides, Saponins, Tannins, Resins and Dialysis.—A. G. D.

Modern Drugs in General Practice, by ETHEL BROWNING. vii + 236 pages. $5^3/_8$ x $8^1/_2$. 1940. Baltimore: Williams & Wilkins Co. $3.00.

This book is intended to bring the general practitioner up to date with regard to the advances in medical sciences made in recent years. It aims to accomplish this purpose by discussing from a therapeutic viewpoint a selected group of drugs which, in the opinion of the author, are the most valuable. The sulfonamide group, gold salts, cardiac drugs, adrenergic and cholinergic drugs, hypnotics and diuretics are among the more important classes of drugs discussed. While the book is intended primarily for the practicing physician, it contains much information on the newer remedies which the pharmacists will find helpful.—A. G. D.

The Manuscript—A Guide for Its Preparation with Instructions for Handling Proof, by SAMUEL E. NORRIS, Compiler. 3rd Edition. xvi + 75 pages. 1941. New York: John Wiley & Sons, Inc. $1.00.

This small volume is intended primarily for authors who are writing for Wiley & Sons, but it contains much information of value to any author. Part I describes preparation and illustration of the manuscript, including copy for the offset process. Part II discusses the handling of galley and page proofs and plate proofs. The book makes no pretense of being a style manual, but it contains practical suggestions on capitalization, punctuation, abbreviations, compounding of words, citation of references and preparation of indexes. It also contains a section on "Poor Usage That Is Common" in which the correct uses of a number of words and phrases are illustrated. The book is believed to be worthy of a place in the libraries of all authors and editors.—A. G. D.

Food Analysis, by A. G. WOODMAN, xii + 607 pages. McGraw-Hill Book Co., Inc., 330 West 42nd St., New York, N. Y., and London, England, 1941. Price, $4.00.

This new edition is an improvement over previous editions in appearance due to the use of a better grade of paper and to wider margins on the page. The book would seem to have use as a brief manual for beginners in food chemistry. The more complex chemical procedures used by food analysts are mentioned by reference only. References to the literature are given at the ends of the chapters as in previous editions. Under food colors, there has been added a discussion of the new permitted dyes, including certain oil-soluble colors. The space given in previous editions to chemical preservatives has been reduced. There has also been added a discussion of the spectrophotometer in its relations to food analysis —A. G. D.